UNIVERSITY OF CAPE COAST

BIOCHEMICAL STUDIES OF RESISTANCE LEVELS OF COCOA VARIETIES AT COCOA RESEARCH INSTITUTE OF GHANA, TAFO-AKIM TO COCOA SWOLLEN SHOOT VIRUS.

BY

DANIEL KOJO MANU

THESIS SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE OF THE SCHOOL OF AGRICULTURE, UNIVERSITY OF CAPE COAST IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE.

MARCH, 2006.

DECLARATION

CANDIDATE'S DECLARATION

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature..... Date 20/03/2007

Name: Daniel Kojo Manu

SUPERVISORS' DECLARATION

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature..... Date 20/03/2007

Name: Mr. Yaw Opoku-Asiama

Co-Supervisor's Signature: ..... Date 20/03/2007

Name: Mr. Patrick Constantine Aculey

ABSTRACT

Biochemical studies were carried out on ten cocoa types to find out their different levels of resistance to Cocoa Swollen Shoot Virus. The beans were inoculated via viruliferous nymphs of mealy bugs, and observed after planting for 3 months. Patches of barks of severe symptom bearing cocoa types were also tested through grafting on healthy Amelonado plants and observed for 3 months. Significant differences were observed in the cocoa types in terms of symptoms appearance and in severity. PA150 X NA33, T63/967 X T65/326 and T85/199 X PA7 were observed to be the best resistant to the CSSVD whilst T85/799 X T65/238, Amelonado X Amelonado, T85/799 X Amelonado, T85/799 XT65/326, and T85/799 X T79/501 were observed to be poor in resistance.

In the investigations, there was weak correlation (0.333) between total polyphenol content and susceptibility of cocoa types to the CSSV, whereas a significant correlation (-0.58) existed between total nitrogen content and susceptibility to the Cocoa Swollen Shoot Virus. PA150 X NA33 had the highest nitrogen content, and statistically different. There were no differences between T85/799 X Amelonado, T63/967 XIMC60 and Amelonado X Amelonado. T85/799 X T65/238 had the lowest and different from the rest.

From the polyacrylamide gel electrophoresis, protein with molecular weights ranged from 12.0 to 62.6 kDa. A pathogenic related protein of size 23.9 kDa was detected in PA150 X NA33, T85/799 X Amelonado and on Amelonado X Amelonado. Another pathogenic related protein of size 46.6 kDa and 23.9 kDa were detected in T85/799 X T79/501.

ACKNOLEDGEMENTS

Many people have contributed to this work from its proposal to the present form but mere specificity is only fair.

Mr. Yaw Opoku-Asiama, my Principal Supervisor, having taken me to Cocoa Research institute of Ghana, Tafo several times for my practical work actually supported me to get the funding and a place for my project work. He also offered invaluable, unparalleled and brilliant suggestions and painstakingly read my project write up to make the necessary corrections and insertions. His special saying "we are not joking here" really spurred me on. I wish therefore to acknowledge with great sincerity my profound gratitude to him.

My Patrick Constantine Aculey, my supervisor, who also became my teacher taught me some basic principles in biochemistry, laboratory instrumentation and above all found time in spite of his busy schedule to read through my work. I wish to say thank you, "jamjam".

The Director, Dr. Michael Roy Appiah and Management of Cocoa Research Institute placed their facilities at my disposal free of charge. They provided me with free accommodation, free access to the laboratories at any time, free medical care, free security, and free recreational facilities, which is very, very rare to find. I therefore thank them for all the things they have done for me.

I am especially indebted to Dr. Yaw Adu-Ampumah, the Deputy Director of Cocoa Research Institute and the coordinator of Common Fund for Commodity (CFC) projects for sponsoring my project. I wish to emphasis that without his

[redacted] this project would not have seen the light of day. I thank him very much for his assistance and also finding time to read through my work.

I wish to thank the Heads of Pathology and Physiology/Biochemistry Departments for allowing me to use their laboratories and for their assistance during the period of my work. I wish to thank Dr. Jimmy. F. Takrama, Dr. Samuel T. Lowor, Dr. Alex Oppong Dwapanyin, Mr. Henry Dzahini-Obiatey, Mr. Michael Asuah; Mr. Joseph Nawo Boafo; Mr. Francis Awuku Osae, Mr. Mawuli Adoblanui, Mr. George Kwaku Danchie; Ms. Gifty Asior Dede, Mr.Benjamin Owusu, Ms. Ophelia Kafui Torsah, Mr. Samuel Ackom, and Mr. Peter Adonteng Boamah-Dankyi, all of CRIG for their support.

Ms. Koranteng Ellen Oye and Mr. Gyamfi Larbi Samuel, both of CRIG library deserve my sincere thanks for getting me all the reference materials from the library and for directing me to the appropriate shelves.

I acknowledge with deep appreciation the financial and moral support of my wife, Livesy.

In mentioning names one may overlook the obvious so I thank all those whose contribution have not been acknowledged. Any credit is theirs.

DEDICATION

To the Glory of God and To My Wife Livesy, My Children, Daniel and Persis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

Cocoa (*Theobroma cacao* L), a crop of ancient origin has become a commodity of major importance in many countries. The importance of cocoa to the world economy is very enormous. To the agricultural sectors of the top ten producer countries including Côte d'Ivoire, Ghana, Brazil, Malaysia; Cameroon, Ecuador, Colombia, Indonesia, Grenada and the Dominican Republic; cocoa is of regional importance, providing a major source of employment in agriculture in these areas (Vingerhoets, 1997).

The crop is consumed widely, mainly in the middle- and high- income countries of the temperate zones. The top 10 countries represent around 75% of world exports (Vingerhoets, 1997).

There is close cooperation between producer and consumer countries, with the objective to reduce fluctuations in prices; to improve the transparency of the world market; to stimulate cocoa development all over the world; and, in general, to work together in all aspects of the world cocoa economy (Vingerhoets, 1997).

West Africa accounts for 68 percent of the world's production; Asia, principally in the Sulawesi area of Indonesia, accounts for 18 percent of the world's total; and, Latin America, in Brazil, Mexico, Ecuador, Costa Rica and a number of Caribbean islands, generating about 14 percent of the world's

supply. These three regions in total produce 3.2 million tonnes of cocoa annually, with a market value of over US $5 billion, (Vingerhoets, 1997).

In Ghana, cocoa has been the mainstay of the economy for decades. As a contributor to the Gross Domestic Product (GDP), in the years 1958 to 1961, it accounted for an average of 13.7 percent of the GDP at constant prices (Killick, 1961). Of the 43% contribution of Agriculture to GDP and 45% of total exports, about 13% of the Agricultural GDP is accounted for by cocoa (World Investment News. Multimedia Information Company. 2002)

Ghana has always been among the first three on the table of producers in the world. For 66 years (1910 to 1977), Ghana retained world's leadership in cocoa production with market shares ranging from 30-40% of the world's total production (Bateman, 1988). Cocoa production has been well above the 300,000-tonnes mark for several years with production peaking at 571,000 tonnes in 1964/65, but has since then fluctuated over the years between 150,000 and 350,000 tonnes per annum as a result of diseases, bad weather and bushfires with Ghana losing her first position to Cote d'Ivoire (Gill and Duffus, 1989).

The uniqueness of the contribution of cocoa to the economy of Ghana lay in the fact that the wealth that it created was rural-based. Cocoa provides employment in many rural communities as well as giving cash to many small farmers. The cocoa farmer did not only produce enough food for the home, but became part of the cash economy through the sale of cocoa. Until recently (1994-95) where the yields of cocoa have gone up to 300,000 tonnes and it is anticipated to increase progressively, yields in cocoa production in the 1980s generally were low and declining because of a myriad of problems

encountered in the industry. General and more localized studies have identified several technical factors which have contributed to the dwindling cocoa production levels in Ghana, Nigeria and other West Africa countries (Adeyimi, 1996, Ollennu *et al.*, 1989; Anon, 1990; Anon, 1995).

Primary among these problems are the ravages caused by insects (cocoa capsids (Heteroptera: Miridae), and diseases such as swollen shoot caused by Cocoa Swollen Shoot Virus and black pod caused by the fungi *Phytophthora palmivora* and *P. megakarya*.

Among all the regional diseases of cocoa, Cocoa Swollen Shoot Virus Disease (CSSVD) is probably of greatest importance (Pereira, 1996), while Thresh (1991) reported that the disease is the most intractable and destructive to strike at the cocoa industry in West Africa. The economic importance is evidenced by the serious decline in Ghana, Togo, Cote d'Ivoire and Nigeria. In Nigeria, large areas have been abandoned due to the devastation by CSSV in areas referred to as 'areas of mass infection' (Adejumo, 2004).

In Ghana the continuous decline in cocoa production has been largely attributed to the incidence of Cocoa Swollen Shoot Disease more than any other disease. Since 1936 several attempts have been made to control the disease. The removal of infected trees and replanting with improved virus tolerance varieties have been the basic method of control for CSSV (Adegbola, 1971). However, Thresh *et al.*, (1988) and Ollenu *et al.*, (1989) have emphasised that successive campaigns of eradication have failed due to the use of cocoa cultivars with moderate tolerance as planting material after eradication.

Biological control by the use of mild train cross protection where cocoa trees were protected using mild strains of the CSSV against more virulent and related strains has been attempted (Adegbola, 1973). This method is risky, for, Broadbent (1964) has pointed out that a 'mild' strain may damage other crops, and may also mutate into virulent strains (Broadbent, 1964).

There have been many previous attempts; both chemical (Mapother and Nicol, 1953; Armstrong, 1961; Hannah and Heatherington, 1957; Marchart, 1968; Firempong, 1984) and biological (Anon, 1951, Decker, 1955; Donald, 1953, 1955), at controlling the mealy bug vectors of the virus but these studies were discontinued for various reasons (Padi, 1997). Recently, studies on the possible use of natural enemies have been revived (Ackonor, 1997).

Biotechnology using the plant tissue culture method for the production of pathogen or virus-free clones of economically important plants is currently being applied in the fight against the Swollen Shoot Virus disease. Studies have been carried out at Crop Research Institute of Nigeria (CRIN) on cocoa micro propagation (including germination) to produce clones as cells, somatic embryos(sexual embryos seeds) without testa *in vitro*) and plantlets (Esan, 1977, 1982) and also *in vitro* germplasm conservation. Also, Adenikinju *et al.*, (1989) reported that in CSSV research, there was recovery of virus-free plants from virus infected indexed cocoa in premium elite stocks. Lately, barrier cropping is being used as one of the control methods against the Swollen Shoot Virus disease in Ghana. It must be realized that an obvious and sustainable solution to the CSSV problem would be to intensify the search for cultivars of cocoa with high level of resistance to CSSV infection.

As a result of various breeding efforts varieties with higher resistance to the virus compared to the standard Amelonado have been developed (Thresh *et al.*, 1988). Adu-Ampomah, (1996) stated that breeding for varieties with resistance to the disease appears more promising.

Out of several germplasm materials assembled in Ghana for use in developing resistant varieties, four cocoa groups namely, the Nanays (NA), the Parinaris (PA), the Iquitos mixed calabacillo (IMC) and the Trinidad introductions (T) which have been utilised successfully elsewhere were investigated in Ghana by Adu-Ampomah, (1996) as sources of better resistance to the disease. However, the levels of resistance in the crosses among these varied cocoa populations were observed not to be consistent.

Some Biochemical and physiological investigations such as carbohydrate metabolism and transpiration in healthy and in CSSV-infected plants have been carried out by earlier workers (Adomako and Hutcheon (1974) Also, reaction of Ghanaian cocoa varieties to CSSV have been conducted to study the disease mechanism of CSSV infection (Thresh *et al.*, 1988). However, very little has been done to use biochemical indexes to assess resistant levels of CSSV cocoa resistant varieties. This study was therefore an attempt to look at the mechanisms of CSSV disease induction at the seedling stage from comparisons of biochemical differences between healthy and infected plants, and cocoa varieties screened for resistance to CSSV, for breeding purposes.

The main objective of the study was to determine the biochemical bases of the variations in resistance levels of cocoa varieties to the Swollen Shoot Virus. In addition, the study attempted to:

(i) study the biochemical changes with respect to phenolic compounds, nitrogen and proteins that take place during infection, and

(ii) study how the biochemical changes influence the type of symptoms that are observed or expressed.

The results of the study would provide a baseline for the studies of factors causing variations in cocoa; establish factors that contribute to resistance in cocoa varieties, and to provide conclusive reasons for the various disease symptoms of CSSV infection.

CHAPTER TWO

LITERATURE REVIEW

Viruses of cocoa

Some of the important diseases affecting Cocoa are those caused by viruses, which have been reported in Côte d'Ivoire, Ghana, Nigeria, Sabah, Sierra Leone, Sri Lanka, and Trinidad (Posnette, 1944; Carter, 1956; Blencowe, 1971; Brunt and Kenten, 1971). In West Africa four distinct groups of viruses have been recognized in naturally infected cocoa. These are the Cocoa Yellow Mosaic Virus (CYMV), reported in Sierra Leone (Blencowe *et al.*,. 1963; Brunt 1970) Cocoa Necrosis Virus (CNV) genus Nepovirus disease observed in Ghana and Nigeria (Owusu, 1971; Thresh, 1958), Cocoa Mottle Leaf Virus (CMLV) in Ghana and Nigeria and Cocoa Swollen Shoot Virus (CSSV) in Côte d'Ivoire, Ghana, Nigeria, Sierra Leone, and Togo. However, only CSSV can be regarded as a major viral disease (Padwick, 1956).

Cocoa Swollen Shoot Virus

Cocoa Swollen Shoot Virus is classified as a badnavirus (Brunt, 1996, Lockhart *et al.*, 1995), a virus with serologically unrelated virions. Cocoa Swollen Shoot Virus has undoubtedly contributed directly and also indirectly to the drastic decline in cocoa production that has occurred in West Africa. Cocoa Swollen Shoot Virus is of great economic importance in West Africa, with numerous symptomatologically distinct variants and a restricted host range. Experimental host range is limited to about 30 species in the

Bombacaceae, Tiliaceae, Sterculiaceae and Malvaceae, some showing differential susceptibility to different strains (Posnette *et* al., 1950; Tinsley and Wharton, 1958). Natural host range is restricted to cacao, *Cola chlamydantha, Ceiba pentandra, Cola gigantea* var. *glabrescens* (Posnette *et al.*, 1950), and *Sterculia tragacantha* (Legg and Agbodjan, 1969).

Cocoa Swollen Shoot Virus strains

According to Adegbola (1971), the virus was named Cocoa Swollen Shoot Virus (CSSV) by the Imperial Mycological Institute in 1946 for the disease was first graft-transmitted by Posnette (1940). The virus had been shown to be a complex of viruses of varying virulence with varying symptoms (Posnette 1947; Posnette and Todd 1955, Thresh and Tinsley 1959). Many strains of CSSV had been reported in literature but four major strains—1A, 1B, 1C, and 1D had been observed in Ghana (Posnette 1947) while 12 strains were observed in Nigeria (Posnette 1947).

Many distinct variants have been recorded; initially named alphabetically; they are now, named by where they were found (Posnette, 1947). The best known Cocoa Swollen Shoot Virus strains include the severe New Juaben strain (strain A; *Theobroma* virus 1A) which is widespread in the Eastern Region of Ghana.

The characteristic features of other well known strains have been recorded by Thresh and Tinsley (1959). These include Bisa, Bosomuoso, Bosumtwe, Mampong, mild New Juaben and Nkawkaw from Ghana; Balogun, Ilesha, Elepo and Offa Igbo from Nigeria, Kongodia and Sankadiokro from Côte d'Ivoire and those from Sri Lanka. Attafuah *et al.*, (1963), also described those strains from Sierra Leone.

Under Smith's Classification (Smith, 1937), Swollen Shoot Virus strain A was termed *Theobroma* virus 1A but by Holmes' terminology, it was *Marmor theobromae* var. A. A combination of scientific, descriptive and local names has become the order. The most prominent isolates intensively studied were the severe strains: New Juaben-1A and Kofi Pare-1A (Group A3), Nsaba-1A and Kpeve (Group A4), and N1, Bisa, SS365B and Bobiriso (Group D).

Physical and biochemical properties of CSSV

In partially purified preparations, the thermal inactivation point is 55-60° C, dilution end point is 10^{-3}-10^{-4} and infectivity is retained without appreciable loss for 2-3 months at 2° C (Brunt *et al.*, 1964; Kenten and Legg, 1965). Crude saps are non-infective but extracts made with antioxidants sometimes infect up to 5% of inoculated cacao beans (Brunt and Kenten, 1962); using concentrated virus preparations, transmission rates from 60 to 90% are obtained

The virions are bacilliform or bullet form, non enveloped, 130nm long and 28nm wide. The genome consists of double stranded, circular DNA with a total genome size of 7.4 kb (Lot *et al.*, 1991).

Mode of transmission of Cocoa Swollen Shoot Virus in nature

A number of badnaviruses occur in clonally-propagated plant hosts and are therefore spread by vegetative propagation of infected plant materials. Some are transmitted in nature by mealy bugs (Homoptera, Pseudococcidae), and several are also seed- and/or pollen-transmitted. Cicadellid leafhopper vectors transmit Rice tungro badnavirus. Mealy bugs are the sole vectors of Cocoa Swollen Shoot Virus (Posnette and Robertson., 1950). Out of 21

species recorded on cocoa in Ghana, about 11 species have been implicated with transmitting the virus from tree to tree (Hall, 1945; Posnette and Robertson, 1950; Roivainen, 1976).

Table 1: Mealy bug species recorded on cocoa in Ghana.

Planococcoides	***njalensis*** **Laing***
Planococcus	***citri*** **Risso***
Ferrisia	***viragata*** **KII +**
Phenacoccus	***hagreavesi*** **Laing ***
Pseudococcus	***concavocerrarii*** **James +**
Pseudococcus	***longispinus*** **Tarq +**
Pseudococcus	***calceolariae*** **Mask**
Planococcus	***celtus*** **Strickland ***
Planococcus	***kenyae*** **Le Pelley**
Planococcus	***ugandae*** **Laing**
Phenococcus	***madariensis Green***
Pseudococcus	***nr. Fragilis*** **Brain+**
Pseudococcus	***gahani*** **Green**
Paraputo	***anomalus*** **Newst**
Paracoccus	***ritchei*** **Laing+**
Paracoccus	***protease*** **Laing**
Dysmicoccus	***brevipes*** **Cockerell+**
Tylococcus	***westwoodi*** **Strickland**
Tylococcus	***boafoensis*** **Strickland**
Tylococcus	***malacanthae*** **Strickland**
Rhizoecus	***spelaeus*** **Strickland**

+ Transmit one or more virus strains

*Transmit all but one of the virus strains

Source: Adu-Ampomah *et al.*, (2002).

Two species *Planococcoides njalensis* Laing and *Planococcus citri* are the commonest and most important (Table 1). The mealy bugs transmit Cocoa Swollen Shoot Virus with varying degree of efficiency (Adu-Ampomah *et al.*, 2002). *Planococcoides njalensis* is the most important vector in Ghana and it transmits most of the virus strains/isolates found in Ghana (Hall, 1945; Campbell, 1983; Bigger, 1981).

Planococcoides njalensis is relatively sedendary (Adu-Ampomah *et al.*, 2002) so other more mobile vectors such as *Phenococcus hagreasi, Planococcus citri* and *Ferrisia viragata* may be relevant in terms of natural spread (Donald, 1955, Campbell, 1983, Bigger, 1981).

The Cocoa Swollen Shoot Virus can occur in many vector species. There is also specificity between individual virus isolates and their vectors. For example CSSV 1M (Mampong) is transmitted by *Planococcoides njalensis* and *Planococcoides adonidum* whereas the close related CSSV 1A (New Juaben) is transmitted by *Planococcoides njalensis* and not by *Planococcoides adonidum* (Adu-Ampomah *et al.*, 2002).

Cocoa Swollen Shoot Virus and vector relationships

Like all badnaviruses, Cocoa Swollen Shoot viruses are transmitted in a semi persistent manner by mealy bug or leafhopper (RTBV) vectors. Nymphs (1 st , 2 nd and 3 rd instars) and female adult mealy bugs spread the virus radially between adjacent trees by crawling through the canopy from infected to healthy trees or being carried by attendant ants (Crematogaster and Camponotus sp.) (Adu-Ampomah *et al.*, 2002). Adult male mealy bugs have only rudimentary non- functional mouthparts and do not feed (Adu-Ampomah *et al.*, 2002) and so the male adults are unable to transmit the virus (Brunt,

1986). Occasionally, jump spread may occur when infective mealy bugs are blown by the wind and infect trees some distance from the original site of infection (Adu-Ampomah *et al.*, 2002; Strickland, 1950; Cornwell, 1960; Thresh *et al.*, 1988).

The mealy bug acquires the virus during feeding on cocoa swollen shoot infected plants. The virus is retained when the vector moults, but does not multiply in the vector nor is transmitted congenitally to the progeny of the insect (Brunt *et al.*, 1996). The vectors can transmit the virus after a 5-minute acquisition feeding, but transmission efficiency increases with longer acquisition feeding. Vectors retain ability to transmit the virus for up to 72 hours and all life stages of vectors can acquire and transmit virus (Lot *et al.*, 1991)

A pre-acquisition starvation period slightly increases vector efficiency, perhaps because it induces the insect to settle more quickly (Posnette and Robertson, 1950). The minimum acquisition feeding period is 20 minute and the optimum is probably 2-4 days (Roivainen, 1969). There is no detectable latent period; insects may transmit within 15 minutes, but maximum transmission occurs after 2-10 hours (Posnette and Robertson, 1950; Tinsley, 1955). The virus persists in feeding insects for 3 hours or less (Posnette and Strickland, 1948), but starved adults and 1st instar nymphs can retain virus for 49 and 24 hours, respectively (Lister, 1953). No transmission occurs through eggs. *Ferrisia virgata* has specifically transmitted all isolates tested except that from Mampong which, like Cacao Mottle Leaf Virus, is transmitted specifically by *Pseudococcus longispinus* (Posnette, 1950).

The use of graft transmission

When healthy Amelonado cocoa seedlings are grafted with bark patches from healthy cocoa or cocoa infected with Cocoa Swollen Shoot, Cocoa Mottle Leaf or Cocoa Yellow Mosaic Viruses, 95% or more of the patches form unions. If the grafts are from cocoa infected with the Ghana isolate of Cocoa Necrosis Virus about 90% of the grafts fail to unite; nevertheless, some 80% of the test seedlings are infected (Owusu and Kenten, 1972). The inoculum pressure has been described in patch grafting as too high and therefore does not stimulate what generally happens in nature during Cocoa Swollen Shoot Virus infection (Adu-Ampomah *et al.*, 2002).

Cytopathology

The virions of CSSV are found in most parts of the plant particularly in the cytoplasm. They occur singly or in large groups, randomly distributed or arranged in palisade-like arrays. They do not occur within inclusion bodies or membrane-bound structures. (Brunt *et al.*, 1995). Most badnaviruses are not tissue-limited, and occur in all tissue types. Apart from changes in the internal organization of mitochondria, there are no data on other cytopathological effects (Brunt *et al.*, 1995)

Diagnostic cocoa species and symptoms

Amelonado cacao is very susceptible to CSSV (Posnette, 1947) and the cacao beans being readily infected by viruliferous mealy bugs (Posnette and Strickland, 1948). The virus can also be transmitted by mechanical inoculation with concentrated virus preparations (Brunt and Kenten, 1962). Seedlings usually produce acute red vein banding and chlorotic leaf symptoms within 20-30 days and, 2-12 weeks later, swellings on shoots and tap roots and

interveinal leaf chlorosis characteristic of the particular strain (Thresh and Tinsley, 1959).

Symptoms of Cocoa Swollen Shoot Virus disease

The Cocoa Swollen Shoot Virus (CSSV) disease produces various symptoms such as red vein banding, vein clearing, and chlorotic spots in specific tissues or organs. Fern pattern and leaf mottle occur in leaves. Swellings occur in roots, on mid-stem swelling and tip of the stem. Mottling occurs on pods of infected plants. These symptoms expressed are different in various cocoa varieties and there is no idea whether the symptoms have anything to do with tolerance or resistance to the virus. The effect of Cocoa Swollen Shoot Virus on the morphology, anatomy, growth and yield of cocoa are well established (Posnette, 1947; Knight and Tinsley, 1958; Dale, 1962; Lockard and Asomaning, 1965). Leaf chlorosis, stem swellings, stunted growth, poor fruiting, die back of shoots and reduced growth of lateral roots are the main symptoms and effects of Cocoa Swollen Shoot Virus Disease reported. According to Thresh (1958), most of the West African isolates of CSSV and some of those of Sri-Lanka produced stem swellings as the only permanent symptoms while others including those from Trinidad showed leaf symptoms. The symptoms may be influenced by other conditions, such as point of inoculation, size of inoculum, and nature of transmission, route of transmission, and physiological and environmental factors. However, their distribution is usually highly specific and often confined to certain organs or tissues. Chlorosis and mosaics only occur in tissues with functional plastids. Malformation such as enations may be confined to veins, while some viruses only affect generative organs (Boss, 1978). Such specific relationships may

be caused by the distribution of the virus within the host. The virus appears to be transported both from cell to cell and along the vascular tracks. Symptoms may arise due to a special sensitivity of certain organs, tissues or organelles to the cocoa virus.

Leaf patterns

The leaf symptoms associated with cocoa viruses were not reported until a few years after the swellings observed by Posnette (1941), though they are often obvious and resemble those caused by viruses in many other plants. According to Posnette (1941) their intensity is probably a better criterion of virulence than is size of swellings. Leaf symptoms usually precede swellings but have not been reported from a few field outbreaks, including the one which occurred near Bisa and others at Peki and Worawora in the Volta Region. Isolates from these outbreaks have been associated with slight or transient leaf patterns in young experimentally infected seedlings. Leaves may become crinkled or asymmetrical with more marked symptoms on the reduced side, depending on the virulence of the strain concerned. Some isolates cause abscission of developing leaves or premature shedding of mature ones, which leads to die-back if repeated on successive flushes (Posnette, 1941).

Almost invariably symptoms are restricted to leaves developed after infection, and with most of the viruses they usually first appear on the young flush leaf soon after emergence. Commonly, the veins are bordered by a narrow red band, caused by accumulation of anthocyanin pigments (Knight and Tinsley, 1958), which in the early stages forms a network over the lamina. Later, the pigment is likely to become restricted to the midrib and lateral veins and portions of the finer veins adjacent to them, giving a red 'feathering'.

With some isolates, such as those from Bosumtwe (Ashanti) and Nkawkaw, the red banding may be wider, the latter sometimes causing a red 'wash' or mottle over much of the lamina, although mainly centred on the lateral veins(Knight and Tinsley, 1958).. This red mottling is even more strongly developed by Kpeve. Reddening usually disappears as the leaves turn green and harden, though it may turn to green vein-banding which sometimes temporarily retains a reddish tint. Some types of cocoa have leaves that are normally deep red in colour when young or have red veins, and it is naturally harder to distinguish the above symptoms on such plants (Knight and Tinsley, 1958). As the leaf enlarges, the red pattern is normally soon joined by chlorotic or transparent lesions, usually also associated in some way with the veins. Vein clearing in the strict sense of the term, when it occurs at all, is confined to the fine veins and normally soon changes to chlorotic vein flecking or banding, the former occasionally being the first symptom to appear on a leaf. Later, chlorosis commonly takes the form of blotching or spotting, sometimes coalescing into bands or blocks (Knight and Tinsley, 1958). These patterns, according to Knight and Tinsley (1958), unlike the reddening, do not disappear as the leaf matures, but may undergo changes until hardening is complete. They observed that such changes could be due to the failure of tissues to develop properly.

Swellings

The first recognized symptoms of virus infection in cocoa were the branch and twig swellings, found in the great majority of outbreaks, from which the name "swollen shoot' arose. These result from greater development of phloem and xylem, both of which are affected by the virus (Knight and

Tinsley, 1958). The swelling is more extensive in the xylem tissue. Xylem vessels tend to occur in clusters, while tracheids and phloem cells are enlarged. (Knight and Tinsley, 1958)

Most Ghanaian isolates produce swellings, which differ in size and frequency between outbreaks. The Kpeve (Cacao Mottle Leaf) virus from the Volta Region, first discovered in 1940, is the best known of those with which this symptom is never associated (Thresh and Tinsley, 1959). Different swelling patterns have been recorded from the Ashanti, the Eastern and Western Regions. Some avirulent isolates of the New Juaben virus seldom, if ever, cause shoot swellings, though the virulent strains produce large and numerous ones. Swellings tend to be more abundant and conspicuous on rapidly growing shoots, for which reason chupons usually bear larger ones in fan branches. They are nodal, internodal or terminal, several often occurring on a single shoot, which may terminate in a dead tip-swelling that ultimately falls from the tree, though further growth may continue from a lateral bud (Thresh and Tinsley, 1959). Even when large and numerous, the swellings themselves do not seem to have much effect on the plant; a Bisa (Krobo District) isolate, though avirulent, produces very conspicuous ones while of two other relatively mild Eastern Region viruses, one (from Mampong, Akwapim District) gives many swellings and the other (from Nkawkaw, Kwahu District), few (Thresh and Tinsley, 1959).

Many isolates produce swellings, with comparable histological abnormalities on cocoa roots; especially the tap-root, on which they may be very large. Some mild strains causing few, if any, swellings above ground induce them more commonly on roots. It has been shown by water-culture

experiments that the size and number of root swellings on seedlings infected by virulent New Juaben are negatively correlated with nitrogen supply (Wharton and Adams, 1955). Plants infected by this and other severe viruses show considerable necrosis of the fine absorbing rootlets.

The effects of Cocoa Swollen Shoot Virus on cocoa plants

Experiments have been carried out to compare the growth of young Amelonado seedlings artificially infected with different isolates. Goodall (1949) showed that, compared with healthy plants, those infected with virulent New Juaben in the bean stage (Posnette and Strickland, 1948) were lower in dry weight, leaf area and growth rate. In general, the dry weights of the component parts were also lower for infected plants; but a smaller proportion of their dry matter was in leaves and lateral roots and a larger proportion in stems and taproots. Infection also caused extensive necrosis of lateral roots, retarded the depletion of food reserves in the cotyledons and reduced the water content of the plants. Many of the effects were apparent within a month of infection and planting Goodall (1949).

In similar observations the effects of nine Ghanaian and four Nigerian isolates were compared (Attafuah and Dale, 1957). A New Juaben one proved the most virulent, reducing total dry weight after eight months to only 16 per cent of that for healthy plants of the same age. Corresponding percentage figures for the others from Ghana were Bosomuoso 28, Kpeve 43, Aiyiboso 45, Mampong 69, Bosumtwe 73, Amafie 76, Wiasi (another Western Region isolate) and Bisa 81 each (Attafuah and Dale, 1957). Most of the plants infected by these two viruses, and some of those with Kpeve, were in a very

poor condition after eight months, and a few had already died (Attafuah and Dale, 1957).

Cocoa Swollen Shoot resistant progenies

Posnette and Todd (1951) after conducting tests on local materials and materials introduced into Ghana concluded that no variety of cocoa is immune to CSSV. Further tests by Longworth and Thresh (1963), Legg and Kenten (1968), and Adegbola (1971) have confirmed the observation of Posnette and Todd (1951). From their studies, Posnette and Todd (1951) noted that some seedlings of Upper Amazon parentage were more difficult to infect with the virus than the local Amelonado, and also some of the tested seedlings developed only slight symptoms, thus exhibiting some degree of tolerance to the infection In the search of tolerant progenies, Longworth and Thresh (1963) found that Parinari and its hybrid clones developed a characteristic necrosis but not true hypersensitivity, while Amelonado, Morona, and Trinitario clones were highly susceptible. They further observed that. Upper Amazon and Scavina clones were less susceptible than Nanay and Iquitos types which were only slightly susceptible. At about the same time, Attafuah and Glendinning (1965a) in Ghana conducted a survey of a progeny of cocoa clone referred to as T17, which had earlier been indicated by Dale (1957) as a resistant clone. This progeny consisted of 52 trees obtained from an open-pollinated pod from Amazon-Iquitos selection IMG 53 in Trinidad, which had been found to be superior in growth and yield to Amelonado and other existing varieties and was then widely distributed. Attafuah and Glendinning (1965a) showed that 47 out of 100 seedlings infected were unaffected by the virus, and out of these,

only one clone, T17 was considered to exhibit an appreciable amount of tolerance.

In another study, Attafuah and Glendinning (1965b) found that clones from Scavina cross unknown progenies were least affected by New Juaben strain, while other Amazon selections, particularly some from Iquitos cross unknown and Nanay cross Iquitos progenies, were very tolerant to infection. They also found that the Parinari and most Trinitario selections were very susceptible.

Kenten and Legg (1970) perfected a simple and very useful method for testing cocoa seedlings for tolerance or resistance to CSSV and CMLV and they used it for testing 45 and 38 progenies in two separate experiments and found resistance among the Nanay, Iquitos, and Scavina, all upper Amazon types, and an Inter-Nanay cross NA 33 X NA 34, while some Amazon-Amazon and Trinitario-Amazon progenies, especially those with T9/21 as parent, were tolerant. T9 had earlier been reported to be the most tolerant Trinitario by Longworth and Thresh (1963), while Toxopeus (1969) also mentioned T9/15 as one of the best clones he tested among Amazon and Trinitario materials.

In another experiment by Kenten (1975) in which progenies were inoculated with 10 viruliferous mealy bugs (twice the usual number) and ranked for resistance on infection levels at first flush showed the strength of the Iquitos selection as a source of resistance relative to the Nanay and the Parinari hybrids. T85/799, Iquitos X Nanay type, T65/238, Iquitos X Parinari type, give percentage infection of 13.4, 22.4, respectively as against T63/967,

T79/501(Nanay X Parinari types) with infection percentage of 26.8 and 29.1, respectively (Kenten, 1975).

Cocoa is an open pollinated (cross breeding) species. This poses difficulties when trying to improve such characteristics as bean quality and yield, or disease and insect resistance in cocoa (Bowers *et al.*, 2001). The genotype T85 is of Iquitos (IMC60) female parent and Nanay (34) male (pollen) parent. T85/799 X T65/238 being Trinidad introductions are however, extremely vigorous (Posnette, 1951; Knight and Rogers, 1955).They come into bearing earlier and are higher yielding than Amelonado and the local Trinitario (Posnette, 1951; Knight and Rogers, 1955).

The genotype T65 is of Parinari (PA7) female parent and Iquitos (IMC47) male parent. T63 is from Parinari (PA35) and Nanay (32) parentage, whereas T17 is from Iquitos (IMC53) and an unknown male (pollen) parent. T79 is of Nanay (NA32) and Parinari (PA7) parentage.

These progenies introduced from Trinidad were promising for vigour, yield and resistance to / tolerance of, the cocoa swollen shoot virus disease and were among the eleven progenies approved for distribution to farmers in 1954 and 1958 for rehabilitating areas devastated by the cocoa swollen shoot virus disease (Thresh *et al.*, 1988). Of the 1944 cocoa introductions from Trinidad, only progenies of Upper Amazons consistently showed higher levels of, resistance to/ tolerance of CSSV than Amelonado progenies (Legg and Kenten, 1968). These included T17/524, T65/238 and T65/ 326.

The Parinaris (PA) are better in terms of yield and black pod disease incidence, PA150 and PA7 than the IMC, NA and Sca. The Parinaris have

higher general combining ability (g.c.a.) for yield and lower g.c.a. for black pod incidence (Adomako *et al.*, 1999a),

Progenies from the Parinaris (PA 7 and PA 35), Nanay (NA31-34), Iquitos Island (IMC47 and IMC60), and Scavina (Sca 12) have been planted extensively in Ghana and elsewhere(Thresh *et al.*, 1988) and have formed the basis of cocoa improvement in many countries for better establishment capacity, vigour, precocity and yield potential than the traditional varieties (Amelonado, Trinitario)(Kennedy *et al.*, 1987; Thresh *et al.*, 1988; Glendinning, 1967; Paulin and Eskes, 1995; Adomako *et al.*, 1999b). The resistance to CSSV of a wide range of cocoa genotypes has been compared by several workers in Ghana and invariably the most resistant were found in the Upper Amazon types (Legg and Kenten, 1968; Kenten and Legg, 1970; Legg and Lockwood, 1977). Of these the Iquitos (IMC) and the Nanays (NA) were the most resistant (Posnette, 1981).

Progenies of Upper Amazon and Trinidad introduction cocoa generally give higher number of beans per pod than the Amelonado and local Trinitarios cocoa progenies (Abdul-Karim *et al.*, 2004). Among the Upper Amazon population, IMC and Nanay families produce pods of higher bean counts than the PA and Sca families (Abdul-Karim *et al.*, 2004).

The dry weight of cocoa is 0.9-1.5 g and the chocolate manufacturers expect that the dry weight of cocoa will be at least 1.0 g (Wood, 1979). Progenies of Upper Amazon and Trinidad introduction cocoa yield beans with dry weight of 1.0-1.2 g. Amelonado and local Trinitarios cocoa produce beans weighing about 1.1g which is similar to that of the Upper Amazons. Among the Upper Amazons population, progenies of Sca usually produce beans less

than 1.0 g whilst IMC progenies yield beans weighing more than 1.1 g (Abdul-Karim *et al.*, 2004) The pod value of cocoa (the number of pods required to produce 1 kg dry weight of beans) varies from 19.0 to 30.0. The IMC and Trinidad introductions give lower pod values than the NA, PA and Sca (Abdul-Karim *et al.*, 2004).

The recommended fat content of Ghanaian cocoa beans is 57.0-58.0 % (Wood, 1979). Beans of Upper Amazon and Trinidad introductions generally have higher fat content (56.0-62%0 than beans of Amelonado and Trinitarios (55.0-57.0%) (Adomako and Adu-Ampomah, 2003).

The Amelonado cocoa is highly susceptible to infection with Cocoa Swollen Shoot Virus (Thresh *et al.*, 1988).It was used as a susceptible control. Amelonado is however, generally resistant to the black pod disease caused by *Phytophthora* species (Wharton, 1959; Glendinning, 1964). The flavour of cocoa is largely determined by the process of fermentation and drying of beans. There is, however, genetic component as Amelonado cocoa consistently gives the best flavour as compared to the other introductions (Thresh *et al.*, 1988).

The conversion ratio of cocoa (the ratio of the dry weight to the wet weight of cocoa beans) ranges from 35.0 to 45 % (Adomako and Adu-Ampomah, 2003). Amelonado cocoa usually has a conversion ratio of more than 40.0% which is generally higher than the values for the Upper Amazon and Trinidad introductions.

Carbohydrate content in infected plant

In their biochemical study of carbohydrate metabolism and translocations in healthy and CSSV infected cocoa plants, Adomako and

Hutcheon (1974) established the accumulation of carbohydrates in the infected tissues. Translocation of photosynthates was found to be equally efficient in the infected and healthy plants Holden (1957).

Mineral content in infected plant

An examination of the data of Lockard and Asomaning (1965) on the mineral content of plants, given adequate nutrients as quoted by Adomako and Hutcheon (1974) showed no difference in the N, P, K, Ca, Mg, Mo and Zn levels of the infected and healthy tissues whilst Fe, Bo, Na, Al, and to lesser extent Mn and Co accumulated in infected plants. Hutcheon (1973) also found no significant difference in water balanced between healthy and infected plants.

The effect of virus on polyphenol content

The production and accumulation of wide variety of organic chemicals is one of the major mechanisms by which plants defend themselves against herbivores and attacks by microbial pathogens and invertebrate pests (Winks and Shimmer, 1999). Flavonoids, terpenes, phenols, sterols, waxes, fats, tannins, gums, suberins, resin acids and carotenoids are among the many classes of compounds known as secondary metabolites (Gottlieb, 1990). Phenolic compounds range from simple phenols (C_6H_5OH) MW 94, found in essential oil of *Pinus sylvestris* to polyphenols such as anthocyanin pigments (MW 2,000) and tannins (MW up to 20,000) (Usher, 1974). Many plant phenolic compounds are polymerized into larger molecules such as proanthocyanidins (PA; condensed tannins) and lignin. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other

natural compounds such as flavonoids, alcohols, hydroxyfatty, acids, sterols and glucosides (Sebelian, 2005)

The anti-viral functioning of polyphenols is very well known (Serkedjieva and Ivancheva, 1999). Guttmann and Feucht (1994) have observed that polyphenols play a vital role in the growth and propagation of plants and protect plant tissue from damage. They further observed that polyphenols neutralise free radicals and thus protect biologically vital molecules from oxidation. Polyphenols are a part of a complex immunity system which can be acquired in tissues under stress (Feucht 1994).

In addition, polyphenols protect plants against insects and herbivorous mammals (Harborne, 1995). Kaur *et al.*, (1989); Kaur *et al.*, (1991) and Baruah and Chowfla (1994) suggested that there are higher contents of polyphenols present in healthy plants. Higher contents of secondary metabolites in healthy plants protect the plants from infection.

On the contrary, Kumar (1991), Sharma and Chowfla (1991) as well as Suresh *et al.*, (1991) stated that there are higher amounts of total polyphenols in virus infected plants. According to Borgo (1991), there were lower amounts of total polyphenols present in the grape cultivars 'Merlot; 'Cabernet Franc' and 'Carbanet Sauvignon', which were infected with the Grapevine leaf roll-associated (3) closterovirus (GLRaV-3) than in those which were not infected and therefore healthy. Later, Guidoni *et al.*, (1997) also discovered lower contents of polyphenols in the skins of 'Nebiolo' grape which was infected with the GLRaV-3 and Grapevine A (?) trichovirus (GVA) than in those which were not infected. The amounts of polyphenols in the grape skins of cv.

'Grignolino' infected with GLRaV-1 and GVA were lower than the contents of polyphenols in the grape skins of healthy ones.

The total polyphenol content in cocoa varies from 12 to 18% (Porter *et al.*, 1991; Rigaud *et al.*, 1993) of defatted and dry weight of the cocoa bean.. The principal compounds are (+)-catechin, (-)-epicatechin and 60% of proanthocyanidins, of which half are dimmers. Other compounds identified are quercetin, quercetrin and p-coumaric, cafeic and chlorogenic acids (Porter *et al.*, 1991; Rigaud *et al.*, 1993).

Nimal *et al.*, (2005) observed that levels of (-)-epicatechin in tea cultivars resistant to blister leaf disease were significantly higher than those in susceptible cultivars, while the reverse was true for (-)-epigallocatechin gallate, suggesting that epicatechin was involved in the resistance mechanism. The content of the methylxanthines, caffeine and theobromine in the leaf increased in the initial translucent stage of the disease, probably as a defence response to fugal attack (Nimal *et al.*, 2005). Epicatechin and epigallocatechin levels were less than in healthy tissues, but increases in the corresponding gallate esters suggested that they were being converted into esters. Although epicatechin and epigallocatechin levels decreased from translucent to mature blister stages, the decrease was not significant (Nimal *et al.*, 2005). The decrease in levels of epicatechin, epigallocatechin and their esters on infection and the formation of cyaniding and delphinidin on oxidative depolymerization of the blisters suggests that proanthocyanidins played a role in the defence mechanism. The high incidence of a purple green leafed cultivar is attributed to the additional catechin source provided by the high levels of anthocyanins present (Nimal *et al.*, 2005).

The total phenolics content of cocoa beans of freshly-harvested pods of different cocoa types were determined by Adomako (1974) in an attempt to find out a correlation between susceptibility in Cocoa Swollen Shoot Virus (CSSV) and the total polyphenols content. The mean value as caffeic acid/g dry defatted cotyledon powder for Amelonado, T85/799 X Sca6/79, T85/799 X T79/501, T85/799 X 1674/N8.122 and T85/799 X P30 were 37, 27.1, 45.2 and 27.6 mg respectively. Comparison with the known order of resistance to CSSV among these cocoa types showed that there was no correlation between the total phenolics content and susceptibility to CSSV.

CHAPTER THREE

MATERIALS AND GENERAL METHODS

The study consisted of laboratory investigations to find out some aspects of the biochemical changes, which occur in cocoa seeds and seedlings infected with severe and mild strains of the Cocoa Swollen Shoot Virus.

The investigations which were carried on the ten cocoa varieties were as follows:

1. Evaluation of symptoms expressed by the ten cocoa varieties inoculated with CSSV strains
2. Determination of total polyphenols content of inoculated cocoa varieties
3. Determination of the changes in Nitrogen content of cocoa varieties infected with Cocoa Swollen Shoot Virus
4. Polyacrylamide Gel Electrophoresis to determine the molecular weight of protein content of cocoa varieties infected with Cocoa Swollen Shoot Virus.

Cocoa varieties

Ten cocoa varieties obtained from Cocoa Research Institute were used for the study. They were considered as the most promising cocoa varieties that combine high yields with resistance to the CSSV disease. They were T85/799 X T65/238, T85/799 X T65/326, T85/799 X PA7/808, T63/967 X IMC60; T63/967 X T17/524, T63/967 X T65/326, PA150 X NA33, T85/799 X

Amelonado (Standard); Amelonado X Amelonado (Self cross) (Standard), and T85/799 X T79/501(Standard).

The varieties were from the various cocoa genotype populations used for the breeding of cocoa varieties resistant to the Cocoa Swollen Shoot Virus Disease. They cover the Trinidad introductions, Nanay (NA), Parinari (PA) and Iquitos Mixed Calabacillo (IMC) collections (Abdul-Karim *et al.*, 2004).

The Trinidad introductions are designated with the letter 'T' and those below T60 were open-pollinated whereas those from T60 and above were hand pollinated (Abdul-Karim *et al.*, 2004).

Table 2 Characteristics of cocoa varieties used for the experiments

Cross	Cocoa type	Characteristics
T 85/799 X T65/238	Inter-Amazons	very/highly resistant to CSSV
T85/799 X T65/326	Inter-Amazons	very/highly resistant to CSSV
T85/799 X PA7/808	Inter-Amazons	very/highly resistant to CSSV
T63/967 X IMC60	Inter-Amazons	very/highly resistant to CSSV
T63/967 X T17/524	Inter-Amazons	very/highly resistant to CSSV
T63/967 X T65/326	Inter-Amazons	very/highly resistant to CSSV
PA150 X NA33	Inter-Amazons	very/highly resistant to CSSV
T85/799 x Amelonado	Series II hybrid	mildly resistant
Amelonado X Amelonado	Amelonado	susceptible
T85/799 x T79/501	Series II hybrid	resistant

The Parents of cocoa varieties designated with the letter 'T' in front e.g. T85/799 (Table 2) are the Trinidad introductions made by Posnette in 1944 (Posnette, 1944). The figure before the slash (85) stands for the pod number of Posnette selection and the '799' after the slash stands for the stand of the tree in the field from which the pods were collected. The Series II hybrids were the first hybrid cocoa released in Ghana by Pound (1943).

The major characteristics of these cocoa types which formed the basis for their selection for the project were their level of resistance to Cocoa Swollen Shoot Virus as indicated in Table 2.

Strains of virus used for the inoculation of cocoa types

The strains of Cocoa Swollen Shoot Virus used for the preparation of source plants for the study were Severe New Juaben 1A virus strain and mild N1 virus strain.

Experiment 1: Assessment of cocoa types for CSSV resistance / tolerance through symptoms expression

The objective was to evaluate cocoa types for CSSV resistance / tolerance through symptoms expression. The experiment consisted of cocoa swollen shoot source plant preparation, the inoculation of the various cocoa types using mealy bugs and patch-graft technique, preparation of soil medium, planting and care of seedlings and finally recording of symptoms and scoring.

Preparation of CSSV source plants

For the study, sources of both New Juaben and mild strains of CSSV were prepared using healthy Amelonado seedlings. The seedlings were obtained from beans of healthy Amelonado pods. The pods were first broken to extract the beans. The pulp was removed from the beans by mechanically

rubbing with sawdust. The testa was then removed with a sharp scalpel. The peeled beans were washed with distilled water and soaked in water overnight, then air-dried (Plate 1) for 4 hours. Adult female mealy bugs (*Planococcoides njalensis*), which were collected from cocoa trees in the field were placed in reproductive cages for 4 days. First and second instars nymphs were transferred onto one-month old Amelonado seedlings (Plate 4) infected with Strain A of Cocoa Swollen Shoot Virus and showing severe symptoms for 72 hours. Some instars nymphs were also transferred onto one month old Amelonado seedlings infected with the mild N1 Cocoa Swollen Shoot Virus strain showing the mild symptoms for 72 hours to acquire the virus (Legg *et al.*, 1972). The nymphs after feeding were used to infect the healthy Amelonado cocoa beans, which were then sown and observed for one month for cocoa swollen shoot symptoms. These infected Amelonado plants were then used as the virus source plants for inoculation of the various cocoa types. After the cocoa swollen shoot source plants were prepared, the various cocoa types were prepared for inoculation and planting.

Plate 1: Cocoa beans air-dried after peeling the testa (x 1 ½)

Inoculations

Two types of inoculations were used: feeding on test plants by mealy bugs carrying the virus and patch graft technique.

Inoculation of cocoa types with CSSV strains using mealy bug vector.

The vectors of CSSV, mealy bugs, were collected from the field daily (Plate 2). The mealy bugs were first cleared of all parasites and debris by brushing them into reproduction cages and kept in desiccators cabinets (Plate 3) and starved for 72hrs. This preservation and starvation in desiccator cabinet were done first to free the mealy bugs of possible contamination of virus acquired in the field and also for them to produce nymphs. Mealy bugs with CSSV loose virus after 72 hours (Lot *et* al., 1991). The nymphs were either fed on CSSV severe strain 1A New Juaben infected seedlings (1A source plants) or on mild N1 infected seedlings (N1 source plants) for acquisition feeding for 72 hours.

Ten nymphs fed on New Juaben strain (1A) were transferred to 90 beans of each of the 10 test cocoa varieties. Again, 10 nymphs fed on a mild strain (N1) were transferred to 90 beans of each of the 10 test cocoa varieties. Thirty of the treated beans were planted in each of aluminium trays measuring 60cm x 30cm.

Plate 2 *Planococcoides* sp. collected from the field (x 10)

Plate 3 Starving of mealy bugs in reproductive cages put into incubator cabinet (x ½).

Plate 4 The feeding of mealy bug on CSSV infected cocoa plant for viral acquisition. The arrow points to the cage containing nymphs of mealy bugs (x ½)

The use of patch-grafting in CSSV 1A strain inoculation

Bark patches from each of the cocoa types infected with Cocoa Swollen Shoot Virus 1A strain were grafted onto healthy Amelonado cocoa seedlings. Twenty seedlings of healthy Amelonado were used for each of the infected cocoa types. In all 200 Amelonado seedlings were used. The symptoms of CSSV on the grafted materials were recorded, one month on the first flush, then the next month on the second flush, and the third month on the third flush.

Soil medium preparation, planting and care of seedlings

The soil medium (black soil) for the nursing of the cocoa types was taken from decomposed refuse dump and heaped at the greenhouse of CRIG. The soil was sieved with a wooden framed sieve of 2mm wire mesh to remove debris and other unwanted materials. The sieved soil was then put into the hollow cylinder, a soil sterilizer, rotated by electrically powered motor and heated. The turning of the tilted barrel allowed for even sterilization of the soil as the heat from kerosene source was applied. The sterilized soil was cooled overnight and used to fill aluminium seed boxes for the nursing of the inoculated cocoa seeds. The filled seed boxes were watered before planting was done and then every other day for four months. The seedlings were observed for symptoms after one month and the observed symptoms were scored as in Table 3

Table 3 Symptoms of Cocoa Swollen Shoot Disease and their score

Symptoms		Score
(i)	Healthy plant with no symptoms of disease	0
(i)	Red vein banding:- Red colourations along the veins (both severe and mild strains). In the case of the mild it disappears from the first leaves as the leaf hardens	1
(ii)	Chlorotic flecking of leaves: Deep patches (big patches) in the leaves (a severe symptom)	2
(iii)	Chlorotic vein clearing/Green vein banding – The veins in the leaves appear as if they are disappearing. (a severe symptom)	3
(v)	Diffused flecking of leaves	4
(vi)	Fern pattern/Swollen Shoot	5
(vii)	Dead plants	6

Source: Adu-Ampomah *et al.*, (2004).

Cocoa Swollen Shoot symptoms scoring and recording.

Recording for symptoms was done one month after planting on the first flush then the next month on the second flush, and the third month on the third flush. The symptoms were scored using a scale 0-6 (Adu-Ampomah *et al.*, 2004) as in Table 3.

Experimental design

The Completely Randomised Design (CRD) was used. There were three factors including (i) viral strains (plants infected with severe 1A virus

strain, mild N1 virus strain and healthy plant), (ii) period, 3 months of symptoms recordings and (iii) varieties, 10 cocoa varieties. There were three replications.

Experiment 2: Determination of total polyphenolics in CSSV infected and uninfected seedlings

The objective was to investigate the amount of total polyphenolic content in healthy and CSSV infected cocoa types and to determine whether there is a correlation between total polyphenolics content and CSSV disease infection. The experiment was carried out in two parts. First, samples of the cocoa varieties were taken and defatted before the total polyphenolics contents were determined.

Defatting of cocoa bean samples by Soxhlet Extraction

For each of the cocoa varieties, 10g of finely ground cocoa bean of each cocoa type was taken and transferred to a porous paper extraction thimble and stoppered with cotton swab. The thimbles with their contents were placed in the extracting chamber of the soxhlets. The soxhlets were fitted onto 250 ml round- bottom flasks each of which was half full with petroleum spirit (60-80^0 C). The set-up was connected to a condenser with the flasks put on a heating mantle. The extractions were done for 18 hours with the soxhlet refluxing several times from the narrow tube (arm) of the soxhlets (Adomako, 1977).

The defatted samples in the thimbles were oven dried at 105^{0}C for about 30 minutes in a Hot Air Oven (Gallenkamp Hotbox Size Two). The samples were cooled in a silica laden dessicator and kept there until required for extraction analysis.

Total polyphenol content determination (tp) Folin-Lowry method (Singleton and Rossi, 1965)

For each of the cocoa varieties 0.2 g defatted cocoa sample was taken for Methanolic HCL (80% MeOH, 1% HCL) extraction during which each sample was shaken in 50 ml volume in plastic vials for 2 hours at room temperature. The samples were centrifuged at 1000 rpm for 15 minutes in a refrigerated centrifuge (Sorvall Rc-5B Refrigerated Super speed Centrifuge).

One millilitre aliquot of the supernatant was used to develop colour reaction with Folin-Ciocalteau reagent (Singleton and Rossi, 1965) (diluted to 0.1 ml sample: 0.9 ml 80% MeOH). To 1.0 ml of the supernatant was added 5.0 ml of Folin-Ciocalteau reagent [diluted 1.0 ml + 9.0 ml in deionised water]. Between 30 seconds and 8 minutes 4.0 mil of 0.075 g/ml $NaCO_3$ reagent was added. The sample was made to stand for one hour at 30^0 C, then later one hour at 0^0 C.

Absorbance reading of sample in 10 cuvette ml was taken at 760 nm with the photospectrometer. Catechin was used to obtain a standard curve (0.2-1.0µg/ml) from which the concentration (g/ml) of the sample absorbance was determined.

The Standard curve (Fig 1) was drawn from a serially diluted catechin. (Singleton and Rossi, 1965).

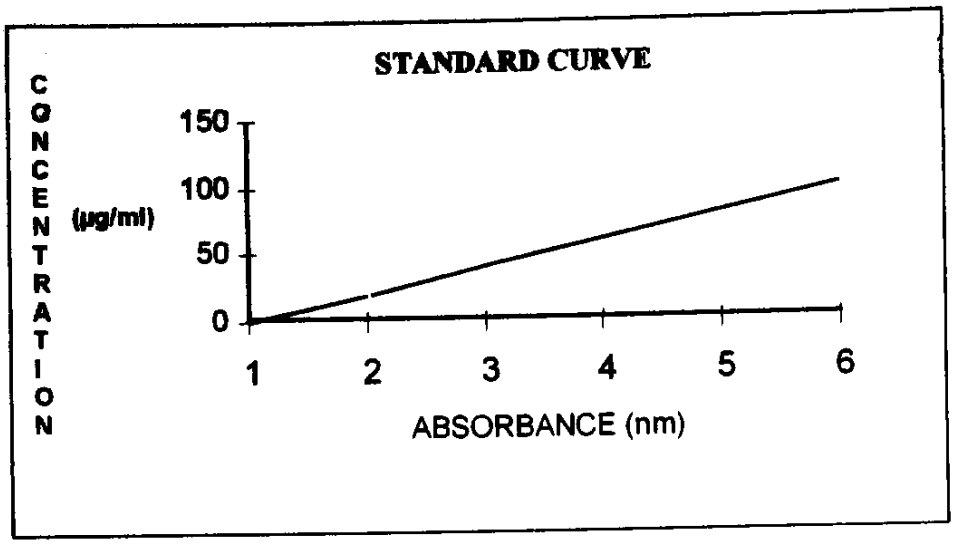

Fig 1 The standard curve from which the concentration of absorbance was calculated.

Experiment 3: Determination of nitrogen in leaves of cocoa types.

This experiment was also carried out in the soil science laboratory of CRIG with the aim of determining the nitrogen (component of protein) levels of infected and healthy cocoa types. In addition the experiment was aimed at determining whether there is a correlation between nitrogen content of cocoa types and Cocoa Swollen Shoot Virus disease infection. The experiment involved the digestion of healthy and CSSV infected leaves of cocoa types.

Digestion of samples of cocoa leaves.

The micro-kjeldahl method as described by Jackson (1964) with modification was used to determine the nitrogen content of leaves of the cocoa types. For each of the various cocoa types 0.5 g finely ground cocoa leaf sample, oven-dried overnight at 80^0 C was weighed into 200 ml kjeldahl flasks.

Ten grams of the catalyst (10g potassium sulphate, 2g copper sulphate and 4g selenium) was added to each sample and thoroughly mixed. Twelve millilitres of concentrated sulphuric acid (Nitrogen free) were added and the mixture was gently heated on a digester with occasional rotation of flask till frothing ceased. The heating was continued until the sulphuric acid began fuming. Further heating was carried out for additional two hours, making sure sample did not stick to the sides of flask. The flask was allowed to cool and 15 ml distilled water added to the sample, and allowed to cool before decanting into a 100 ml standard flask.

The digestion flask was rinsed three times with 20 ml of distilled water, each time adding the washing to the standard flask. The solution was cooled to room temperature and the solution topped up with distilled water to the mark.

A 10 ml aliquot of the solution of each of the varieties was transferred into the Markham distillation flask. To this 5 ml of 40% sodium hydroxide solution was added. The sample was mounted on the Markham distillation apparatus and distilled into a 100 ml conical flask containing 20 ml of 2% boric acid which contained screened methyl red indicator (0.1g methyl red and 0.1g methyl blue in 50ml of 95% alcohol). Ten millilitres of the distillate containing nitrogen were titrated against standardized 0.025N H_2SO_4, till the end point was reached. The nitrogen content in the leaf samples of the cocoa types was calculated with the titre value obtained from the titration by the formula as follows:

Percentage Nitrogen in the leaf sample,

$$\%N_2 = \frac{T \times 0.02}{\text{Weight of sample}} \times \frac{14}{1000} \times 10 \times 100$$

T=Titre value, 0.02 = Normality of acid, 14/1000 = 1 Normal or molar of Nitrogen in solution; 10 = Aliquot of digested sample taken, and 100 = Results expressed as a percentage.

1ml of 0.02N H_2SO_4 = 0.28 ml (Ammonium Nitrogen)

$$\%N_2 = \frac{0.28 \times 0.02N\ H_2SO_4 \times 10 \times 100}{1000 \times 0.5g} \times 0.56 \text{ (leaf sample)}$$

$$\%N_2 = \frac{T \times 0.02 \times 14}{0.5g} \times 0.56$$

Experiment 4: Determination of the molecular weight of proteins synthesized in infected and uninfected cocoa varieties by polyacrylamide gel electrophoresis (PAGE)

In this experiment, SDS-PAGE was performed as described by Andrews, (1986). It was used to determine the molecular weight of proteins synthesized during infection. A low molecular weight standard run on a 12% polyacrylamide gel stained with Coomassie R250 was used. The objective was to determine the apparent synthesis of proteins in both healthy and Cocoa Swollen Shoot Virus infected Cocoa and also to determine how the molecular weights could be used to assess CSSV infection in cocoa types. Proteins from leaves of each the cocoa type were first extracted and kept in a freezer. The casting gel apparatus assembled before the preparation of the resolving and

stacking gels, (Laemmli, 1970) and the subsequent loading and running of the protein samples.

Extraction of protein samples

For each of the cocoa varieties, 0.4g of leaf sample was taken from healthy cocoa varieties as well as those infected with both severe New Juaben Cocoa Swollen Shoot Virus strain (1A) and a mild strain (N1). The samples were ground in liquid nitrogen with 0.8 g insoluble Polyvinylpirolidine (PVP) in a mortar. The powder from the various samples was transferred into different beakers containing 9.5 ml extraction buffer (50 mM pyridine, 10 mM Theourea, 1% SDS) and stirred for 15 minutes with a stirrer.

The samples were transferred into polycarbonate centrifuge tube and clarified at 16000 rpm for 40 minutes at 5°C. Cold acetone was added to the clarified samples to a final volume of 90% and stored in a freezer at -20°C for 2 hours to precipitate. The samples were centrifuged at 9000rpm for 20 minutes to collect the precipitates (proteins). The supernatants were discarded and the precipitates were washed again with cold acetone and centrifuged at 9000 rpm for 20 minutes. The proteins in the form of pellets were dried in a vacuum for 30 minutes and re-suspended in 1ml of the extraction buffer and stored at -20°C for PAGE. (Dzahini-Obiatey *et* al., 2002)

The separating gel preparation

The gel apparatus was set up in stages (Fig. 2, Fig. 3 and Fig. 4), and a gel matrix consisting of polyacrylamide (for proteins) was prepared. A polyacrylamide gel containing acrylamide that are cross-linked to each other using bis-acrylamide was prepared.

An amount of 2.5 ml of 1.5M Tris-HCL, (121g/L and 18.8/L) at pH 8.8 was added to 3.35 ml of distilled water and 0.1 ml of 10 %(w/v) SDS was then added followed by 4.0 ml Acrylamide/Bis-acrylamide (30%/0.8% w/v).This formulation used an acrylamide stock of 29.2 % acrylamide and 0.8 % bis-acrylamide, the cross-linker (cross linking gives the gel its mechanical stability). To initiate polymerization, 0.05 ml of freshly prepared10% ammonium persulphate was added to the mixture An amount of 0.005 ml N, N, N', N'-tetramethylethylenediamine (TEMED) was added giving a total volume of 10.005 ml just prior to pouring (Fig 5) using a Pasteur pipette and a rubber bulb. The gel was allowed to polymerize before stacking gel was added by overlaying gently with butanol. The butanol was added to produce a smooth, completely level surface on top of the separating gel, to make the bands straight and uniform. Polymerization was confirmed by sucking some of the remaining gel mixture into the pipette, allowing it to stand, and checking it after 10 minutes. When squeezing the bulb could no longer expel the gel mixture, the separating gel was set.

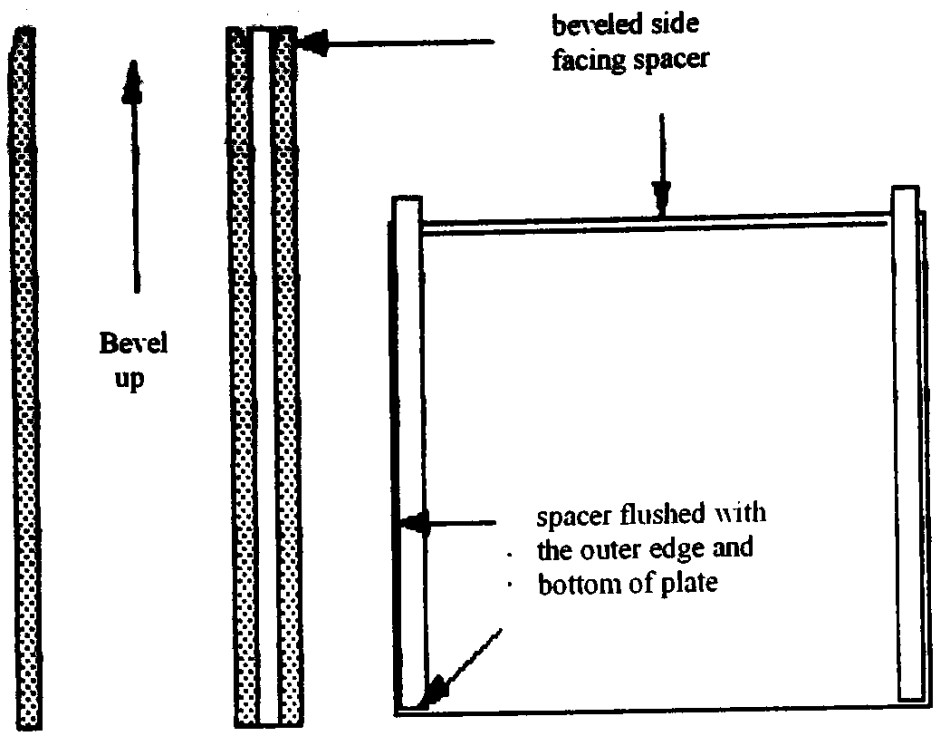

Fig 2 A single gel cassette, properly assembled (× 1).

The stacking gel preparation

After the separating gel had polymerized, a 4.0% stacking gel was prepared. An amount of 2.5 ml of 0.5M Tris-HCl, at pH 6.8 was added to 6.1 ml of distilled water. To this mixture, 0.1 ml of 10 % (w/v) SDS was added followed by the addition of 1.3ml Acrylamide/Bis-acrylamide (30 % / 0.8% w/v) and 0.05 ml of freshly prepared 10 %(w/v) ammonium persulphate (AP) was added to initiate polymerization followed immediately by the addition of 0.01 ml N, N, N', N'-tetramethylethylenediamine (TEMED). Before the final two components were added, which would start polymerization, the butanol was poured off the separating gels into a sink with the tap water running and excess butanol/acrylamide removed with a pipette. After AP and TEMED were added, the mixture was immediately swirled and poured into cassettes

(Fig 2) on top of the gel plates (Fig 5). The stacking gel was poured between two glass plates spaced 1mm apart up to 1cm from the top of the plates. A thin layer of butanol was carefully layered over the top of the stacking gel so it does not dry out while polymerization was occurring. Combs were inserted one at a time (Fig 6) into the stacking gel before it hardened, with care taken not to catch bubbles under the teeth, and adjusted to make them even by scraping excess stacking mixture off later. After the stacking gels had hardened, the combs were removed, leaving well-defined sample wells. The purpose of the stacking gel was to allow the proteins in the samples that were applied to the wells to form tight narrow bands before entering the resolving gel where the separation occurred when they were run.

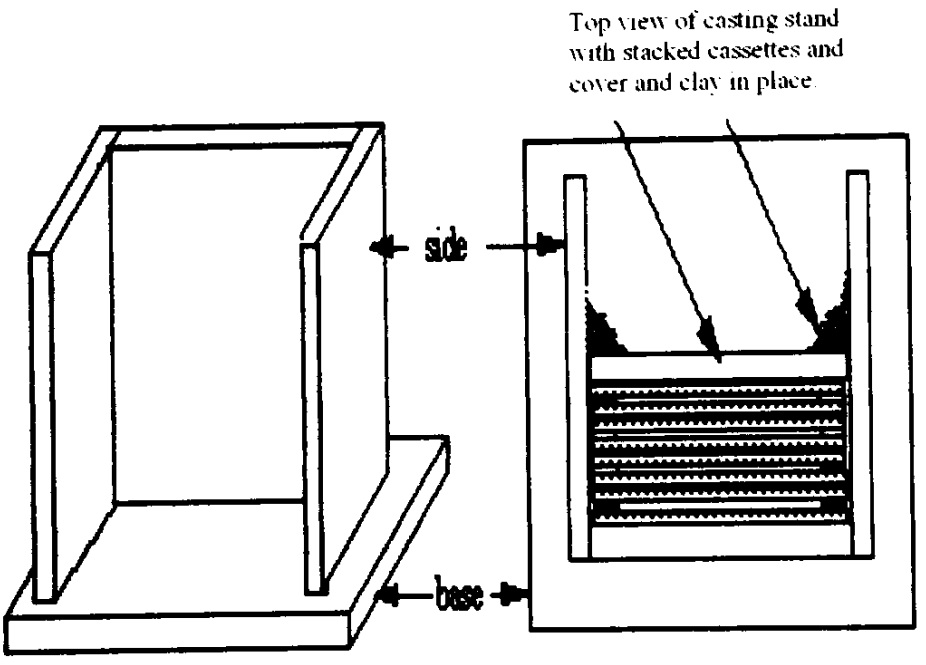

Fig 3 Empty casting stands showing stacked cassettes (× ½).

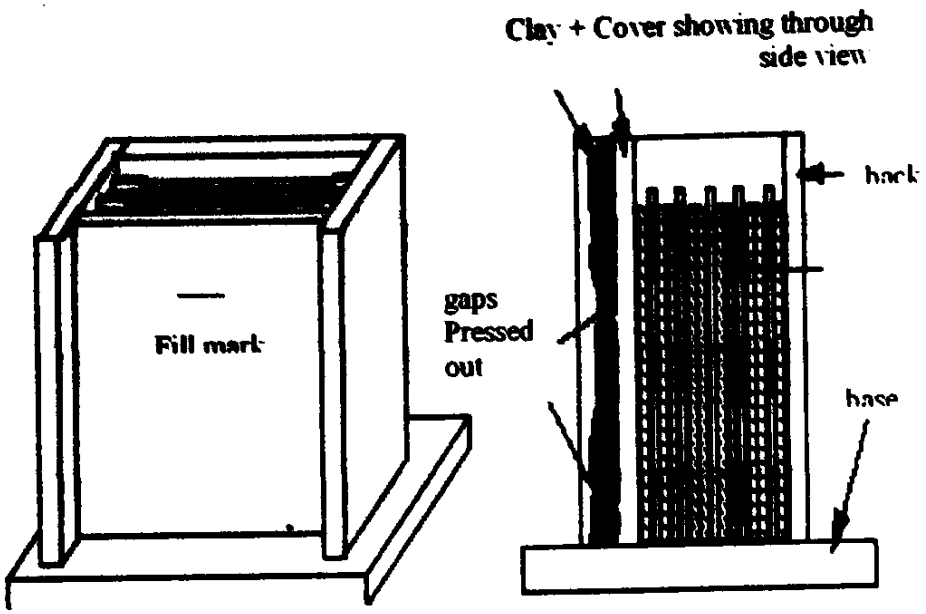

Fig 4 A complte bio rad Protean II casting stand ready for casting gels and loading of samples (× ½).

Source: (Bio Rad laboratories, 1987)

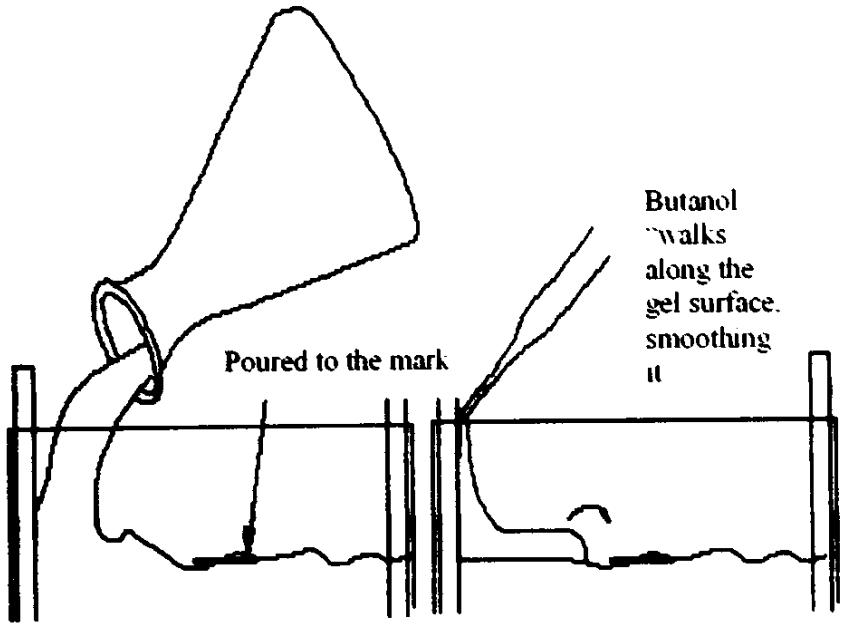

Fig 5 A set up showing the casting of gels (× ½).

Source: (Bio Rad laboratories, 1987)

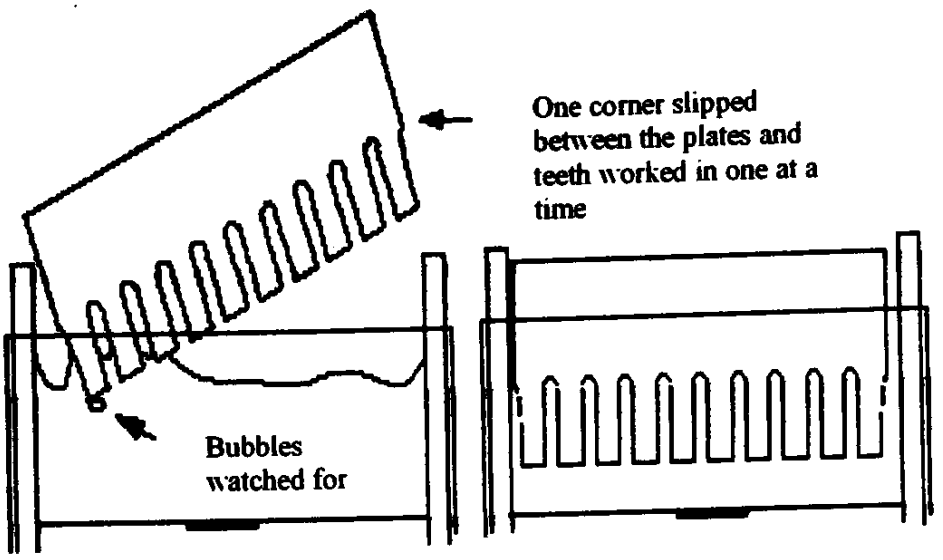

Fig 6 A set up showing the creation of wells for loading of protein samples (× ½).

Source: (Bio Rad laboratories, 1987).

Protein samples preparation and loading.

Fifteen millilitres sample buffer was prepared with 1.88 ml of Tris buffer pH 6.8, 6.0ml of 10% SDS, 3.0 ml Glycerol; 2.31 mg dithiothreitol, and 20 mg Bromphenol blue and topped up with water. Hamilton syringes were used for loading samples into the wells. Twenty micro litres of each of the extracted cocoa samples and 10 (5 ml 2-mercaptoethanol / 50 ml buffer) micro litres of sample buffer were taken and loaded into the wells at a predetermined order in different plates. The plates were labelled (A-D). The glycerol in a sample caused it to sink neatly to the bottom of the well, allowing as much as 20 µl or even more to be loaded. In addition to the proteins, the sample mixture also contained a low molecular weight dye, the bromphenol blue, which migrated ahead of most protein samples that were being separated. The

migration of the proteins on the gel was invisible, but the migration of the dye could be easily followed.

Running gels and processing of protein samples.

After the gels were prepared, they were placed in an electrophoresis apparatus. The apparatus appeared to be complex, but it actually performed a very simple function by applying a current that run through the gel. The gel was placed between two buffer chambers. The upper buffer chamber contains the cathode and the lower chamber contains the anode.

The samples were placed in the wells, and the apparatus was connected to a power supply and current applied. The gels were run at 200 volts for 45 minutes so as to track dye to the bottom as quickly as possible without overheating the gels. Overheating could distort the acrylamide or even crack the plates. The gels were run in 300 ml electrode buffer (10 X running buffer, pH 8.3: 30.3 g Tris, 144.2 g Glycine and 10 g SDS all in 1litre of water)

Negatively charged proteins migrated toward the anode. In the process of running, the proteins were denatured by boiling with sodium dodecyl sulphate (SDS) and 2-mercaptoethanol. SDS is a detergent that binds tightly to proteins, normally in a ratio of about one SDS molecule for every two amino acids in a protein. SDS is negatively charged, therefore, when it binds to all the proteins in a mixture, it gives them a charge that is roughly proportional to its weight. All the negatively charged proteins migrated toward the anode at the base of the gel. However, their migration was impeded by the cross-linked polyacrylamide polymer, which formed a molecular sieve. Larger proteins migrated more slowly than smaller proteins. When the dye band was within 2-

3 mm of the bottom of the gel, migration was stopped by turning off the electrical field.

Disassembling and staining of protein gels

When the run was stopped, the gels were removed from the cassette by separating the plates. The gels were stained as described by Blum *et al.*, (1987) where they were placed into a staining dish containing deionised water. After a quick rinse, the water was poured off and stain added. Staining was done by incubation overnight, with agitation. The agitation circulated the dye, facilitating penetration, and helped ensure uniformity of staining. A commonly used stain for detecting proteins in polyacrylamide gels was used, that was 0.1% Coomassie brilliante Blue dye in 50% methanol, 10% glacial acetic acid. Acidified methanol precipitates the proteins.

The dye actually penetrated the entire gel; however it only stuck permanently to the proteins. Excess dye was washed out by 'distaining' with acetic acid/methanol, (70 ml/200 ml and 730 ml water) also with agitation.

The stained/distained gels displayed a pattern of blue protein bands against a clear background which were photographed, analyzed and documented.

The molecular weights of the proteins of the samples were determined by calculation with the formula

Data analysis of protein gel electrophoresis

Since the charge on proteins is roughly proportional to size, it was possible to estimate the molecular weight of proteins by SDS-PAGE. Unknown samples were run individually and a series of standards were also run in a single well. After the protein bands were fixed and stained, their R_f values were

determined by dividing the total distance they migrated by the distance migrated by the bromphenol blue dye (Weber, K. and Osborne, M. 1969).

$$R_f = \frac{\text{Distance migrated by protein}}{\text{Distance migrated by dye}}$$

A Plot of the log MW verses R_f for the standards was made.

Table 4 Molecular weight of standards

SDS-PAGE Standard	MW and references
Phosphorylase B	97,400 (Titani *et al.*, 1977)
Serum albumin	66,200 (Brown, 1975)
Ovalbumin	45,000(Warner, 1954)
Carbonic Anhydrase	31,000(Davis, 1971)
Trypsin Inhibitor	21,500(Wu and Scheraga, 1962)
Lysozyme	14,400 (Jolles and Angew, 1969)

The molecular weights of the standards are listed above at the right side in Table 4. Since this was a reducing SDS-PAGE gel, these were actually the subunit molecular weights for any of the proteins which contained multiple subunits. The molecular weights of the major bands in the samples were determined using their R_f values and the prepared plot.

CHAPTER FOUR

RESULTS

General observation of studies on the Cocoa Swollen Shoot Virus symptoms

The cocoa varieties inoculated with the Swollen Shoot Virus 1A strain by the mealy bug showed red vein banding of leaves, chlorotic vein flecking of leaves, chlorotic vein clearing of leaves and green vein banding (Plate 5-7). T85/799 X Amelonado, Amelonado X Amelonado and T85/799 X T79/501 were the only progenies which showed fern patterns of leaves (Plate 8). Only one plant (Amelonado X Amelonado) showed the swollen shoot (Plate 9) symptom out of a total of 600 plants used for the entire experiment. The patch-grafted seedlings however, showed all the symptoms as well as chlorosis of the leaves (Plate 10) of the mealy bug inoculated seedlings

The red vein banding of leaves was the first symptom observed. In some of the plants the symptom vanished after a day. In others, however, they persisted for over four days. The disappearance of the red vein banding was followed immediately with the hardening of the leaves. For some plants red vein banding was latent throughout the study period of three months.

Generally, the disease symptoms progressed from red vein banding to chlorotic flecking of leaves then to chlorotic vein clearing and green vein banding. Fern patterns of leaves were observed on some of the seedlings inoculated with mealy bugs on the third month, however, all the patch-grafted

seedlings showed this symptom by the second month. T85/799 X T79/501, T85/799 X Amelonado and Amelonado X Amelonado recorded the highest death of patch grafted seedlings representing 55, 45 and 40 % respectively. The chlorotic patch-grafted plants (T85/799 X T17/501) eventually died.

T85/799 X T65/326 and T85/799 X PA7/808 did not record any death of grafted seedlings. T63/967 X IMC60 recorded 10% death, T63/967 X T17/524, 30% T63/967 X T65/326, 35% and PA150 X NA33, 10%. Inoculated plants were consistently compared to the control plants in Plates 11 to ensure correct identification of disease symptoms.

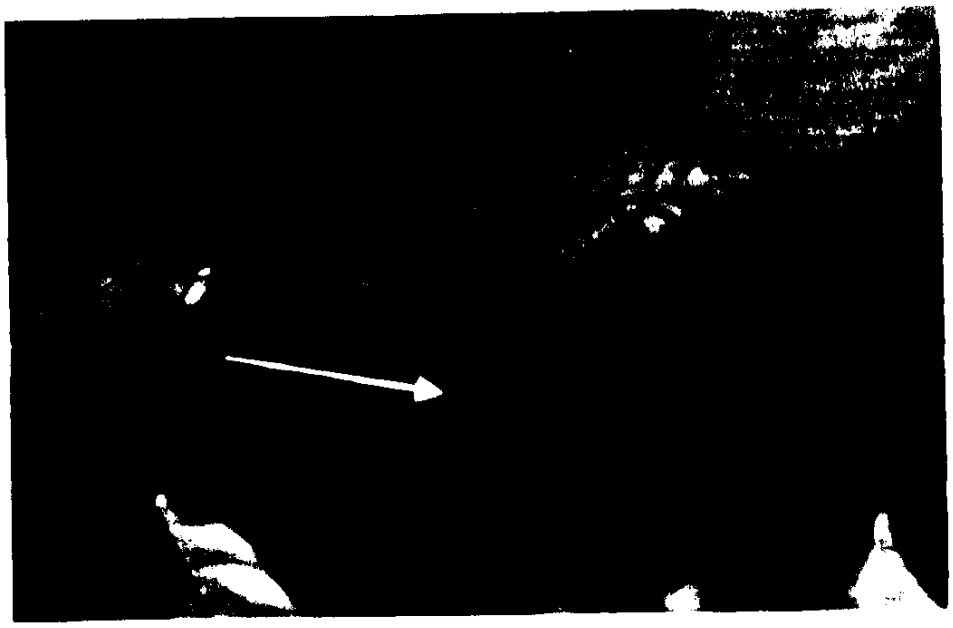

Plate 5(a) Red vein banding, a mild Cocoa Swollen Shoot Virus disease symptom of mealy bug inoculated seedling (× ½).

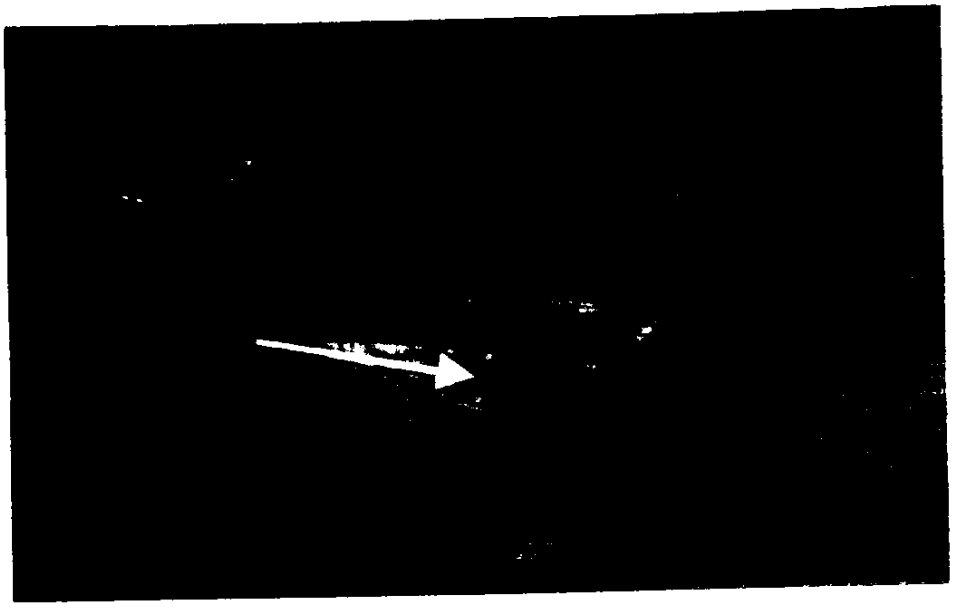

Plate5(b): Red vein banding, a mild Cocoa Swollen Shoot Virus disease symptoms of graft inoculated seedling (× ½)

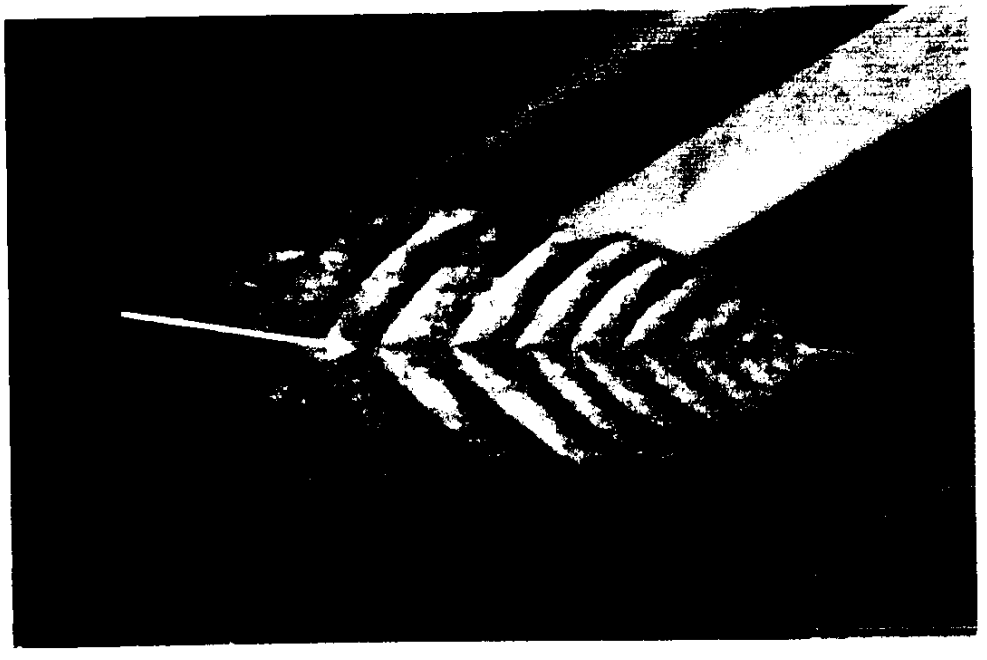

Plate 6 Chlorotic flecking of leaf. This is a mild symptom (× ½)

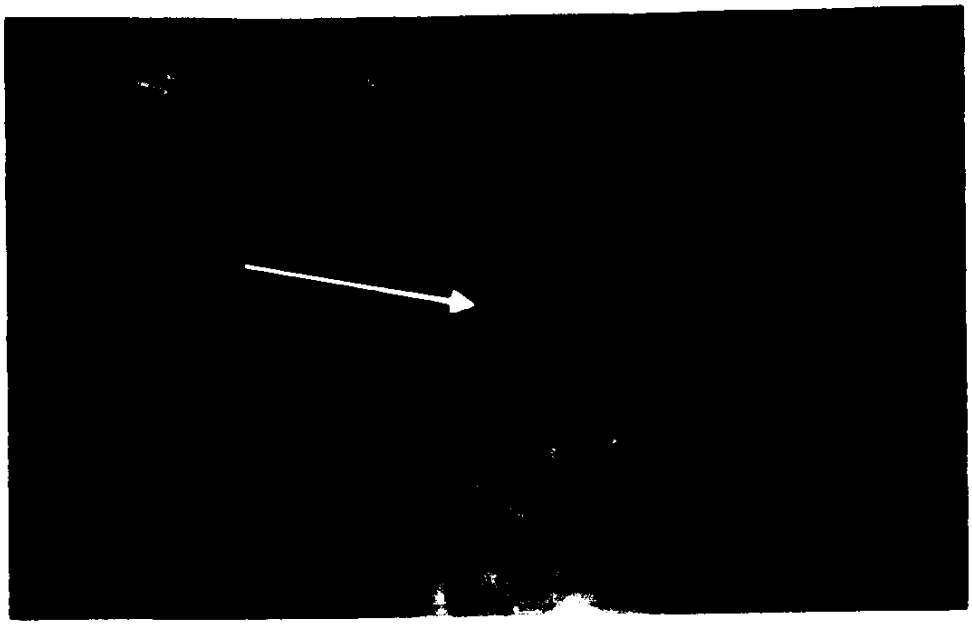

Plate7 Vein clearing and green vein banding of leaves. Both are severe symptoms (× ½).

Plate 8: Fern pattern of leaves. This is a very severe symptom (× ½).

Plate 9 The Swollen Shoot symptom. This is also a very severe symptom.

(× ½).

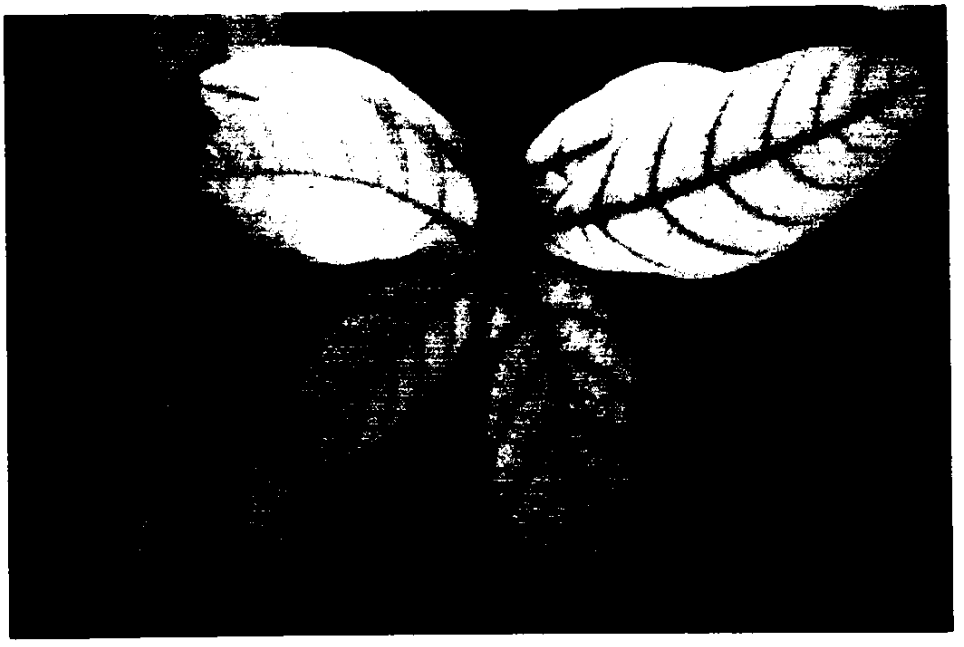

Plate 10 Chlorosis of leaves observed on T85/799 X T79/501 inoculated by patch grafting (× ½).

Plate 11 Healthy cocoa plants, three months after planting used as standard in laboratory work (× ½).

Mean number of Cocoa Swollen Shoot Virus disease symptoms

From Table 5, the number of Cocoa Swollen Shoot Virus disease symptom showed significant differences at probability level of 0.01. PA150 X NA33 had the least value of 28.17. The highest symptoms were observed on T85/799 X T65/238, followed by Amelonado X Amelonado. The value of 33.83 observed on T85/799 X T65/326 was significantly not different from those observed on T85/799 X AMEL (STANDARD) and T85/799 X T79/501 (STANDARD) (Table 5). In the same way, the value of 30.22 observed on T85/799 X PA7/808 was also significantly not different from that observed on T63/967 X T65/326. T63/967 X IMC60 and T63/967 X T17/524 were significantly different from each other (Table 5).

Table 6 Number of Cocoa Swollen Shoot Virus symptoms observed on cocoa types

Treatment (Cocoa types)	Symptoms observed
T85/799 X T65/238	42.11 a
AMEL X AMEL (STANDARD)	35.28 b
T85/799 X T65/326	33.83 c
T85/799 X AMEL (STANDARD)	33.72 c
T85/799 X T79/501 (STANDARD)	33.72 c
T63/967 X IMC60	33.22 d
T63/967 X T17/524	32.72 e
T85/799 X PA7/808	30.22 f
T63/967 X T65/326	30.17 f
PA150 X NA33	28.17 g

**Significant at P=0.01. LSR value = 0.4745 ± S.E =1.19

Means followed by a common letter are not significantly different from each other.

Incidence of Cocoa Swollen Shoot Virus disease on seedlings inoculated with mealy bugs and by patch grafting.

Figure 7 drawn with the data from Appendix 5 shows the results of the incidence of Cocoa Swollen Shoot Virus disease on both seedlings inoculated with mealy bugs and those inoculated through patch grafting from the first

flush to the third flushes. Highly significant differences exist between the patch grafted seedlings and seedlings inoculated with mealy bugs. Highly significant differences also exist between the first and the second flush and the first and third flushes, in both seedlings inoculated with the mealy bug and the patch grafted seedlings. However, there is no difference between the second and the third flushes in both seedlings inoculated with the mealy bug and the patch grafted seedlings. PA150 X NA33 had the highest incidence for both seedlings inoculated with the mealy bug and for the patch grafted seedlings whereas T85/799 X T79/501 (STANDARD) had the lowest incidence of the CSSV disease for only the patch. grafted seedlings.

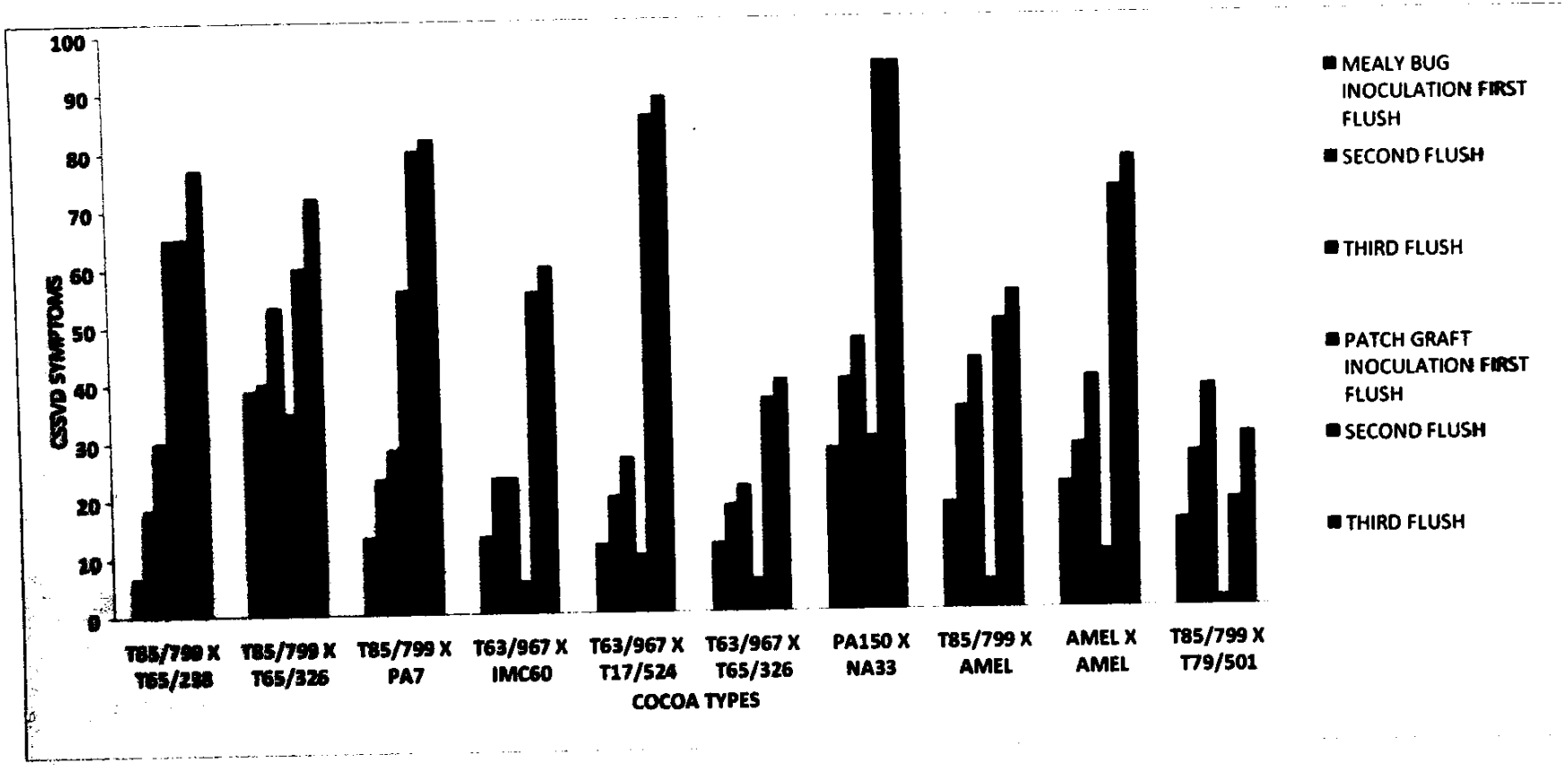

Fig 7: Histogram of incidence of Cocoa Swollen Shoot Virus disease in mealy bug inoculated seedlings and patch grafted Seedlings.

****Significant at P= 0.01. LSR value = 0.4745.**

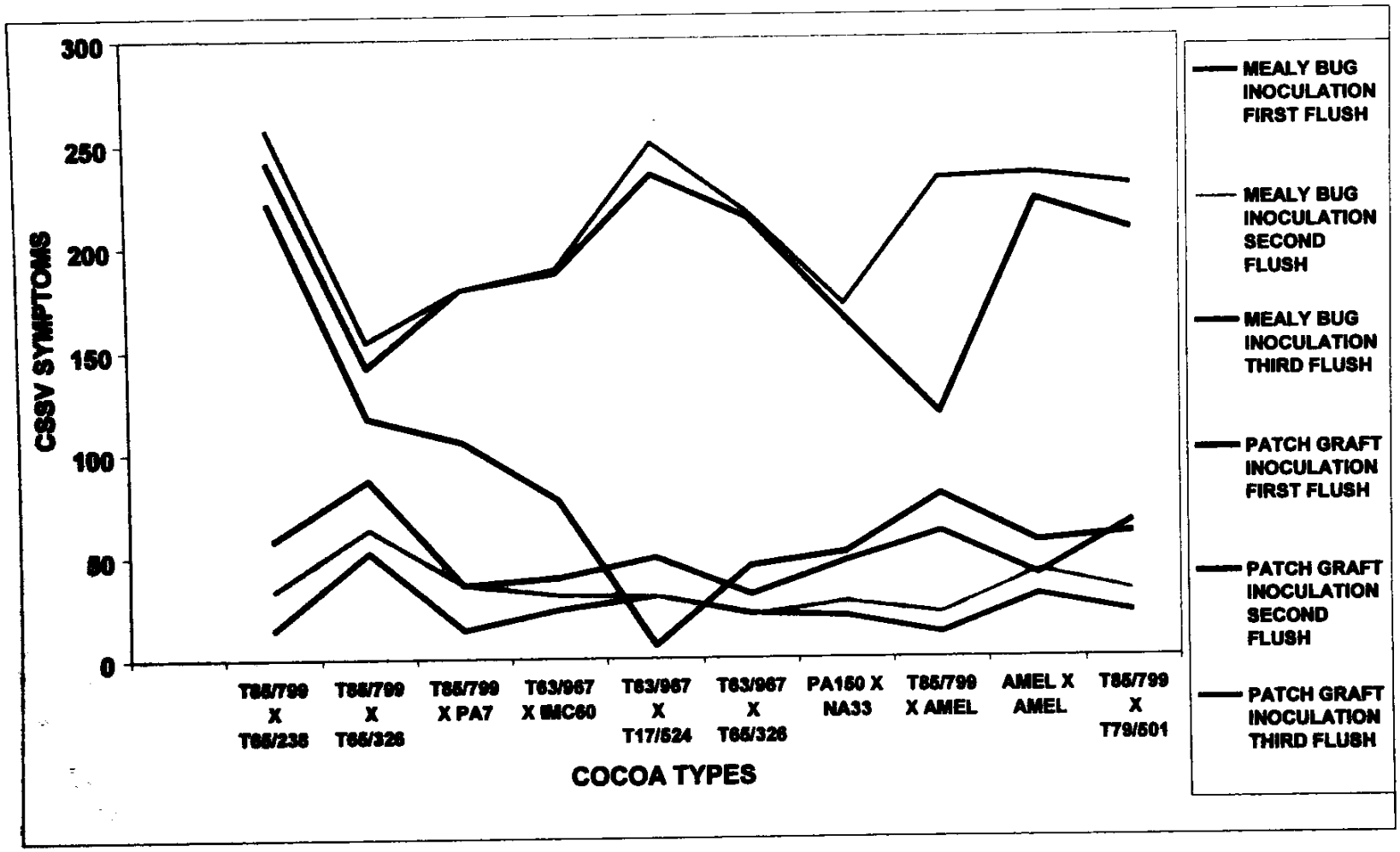

Fig 8 Interaction between cocoa types and methods of inoculation in severity of Cocoa Swollen Shoot Virus disease.

**Significant at P=0.01. LSD value = 0.4745.

Fig 8 showed the extent of severity of CSSV on the various cocoa types and the interactions of the methods of inoculation. There was significant difference between the mealy bug inoculation and the patch grafted seedlings in terms of CSSV severity. The grafted seedlings were most affected. Amelonado X Amelonado and T85/799 X T85/238 were the most affected by the virus with no difference between them through the graft inoculation. PA150 X NA33 had the lowest severity level of CSSV infection, followed by T85/799 X T65/326. T85/799 X PA7/808 was better in terms of having low severity level of CSSV infection than T85/799 X Amelonado so was T85/799 X Amelonado better than T63/967 X T65/326. Even though T63/967 X T17/524 had the lowest infection rate, during the first flush, by the second and third flushes, the severity rate had gone higher than the rest and it is only lower than T85/799 X T79/501 and the other two cocoa types which recorded the highest level of severity.

Total polyphenolics content of cocoa types

As shown by the data in Table 6, there were highly significant differences between the total polyphenolics content of the various cocoa types. There were highly significant differences recorded between the cocoa types. The three standards used recorded the highest total polyphenol contents. T85/799 X T79/501 (STANDARD) had the highest mean content of total polyphenols of 32.25 g/kg. The lowest total polyphenolics content was recorded by T63/967 X IMC60 (17.09 g/kg).

Table 6 Mean total polyphenolics content of cocoa types

Treatment (Cocoa type)	Total polyphenolics (g/kg)
T85/799 X T79/501 (STANDARD)	32.25 a
AMEL X AMEL (STANDARD)	30.86b
T85/799 X AMEL (STANDARD)	25.84c
T85/799 X T65/326	25.47d
PA150 X NA33	23.00e
T63/967 X T17/524	22.27f
T63/967 X T65/326	21.74g
T85/799 X PA7/808	19.97h
T85/799 X T65/238	19.54i
T63/967 X IMC60	17.09j

**Significant at P=0.01. LSR value = 0.02172 ± S.E. 1.54

Table 7 showed statistical differences in total polyphenol content in the interaction between variety and virus strain. Significant differences exist between the strains used and also between the cocoa types. CSSV 1A infected plants recorded a significantly higher amount of total polyphenols than the healthy control plants, which was also different from the amount produced by N1 infected cocoa types. Amelonado X Amelonado produced a significantly different total polyphenols in the healthy plant than the other cocoa types whilst PA150 X NA33 recorded a significantly lower amount. T85/799 X

T79/501 recorded a significant amount of total polyphenol content after infection with the CSSV 1A strain whilst T63/967 X IMC60 recorded the lowest quantity of total polyphenols. Amelonado X Amelonado and T85/799 X Amelonado were significantly not different from each other in the amount of total polyphenols produced in the N1 infected tissues.

Table 7 Total polyphenols content (g/kg) of cocoa variety/ CSSV strain interactions

Variety (cocoa types)	1A strain	N1 strain	Healthy control
T85/799 X T79501		42.45 a	
AMEL X AMEL	40.15 b		
T85/799 X T65/326	36.83 c		
T85/799 X T79501	36.72 d		
AMEL X AMEL			35.74 e
T63/967 X T17/524	35.58 f		
T85/799 X AMEL	34.52 g		
PA150 X NA33		32.73 h	
T85/799 X T65/238	27.25 i		
T85/799 X PA7/808	26.80j		
T85/799 X T65/326			26.73 k
T85/799 X AMEL			26.32 l
T63/967 X T65/326	24.90 m		
T63/967 X IMC60			23.37 n
PA150 X NA33	23.07 o		

[illegible] of cocoa variety/ CSSV strain interactions (continued)

Variety (cocoa types)	1A strain	N1 strain	Healthy control
T85/799 X PA7/808			22.31 p
T63/967 X T17/524			21.51 q
T63/967 X T65/326			21.36 r
T85/799 X T65/238			20.90 s
T63/967 X T65/326		18.94 t	
T63/967 X IMC60	17.60 u		
T85/799 X T79/501			17.58 v
AMEL X AMEL		16.67 w	
T85/799 X AMEL		16.67 w	
PA150 X NA33			13.20 x
T85/799 X T65/326		12.84 y	
T85/799 X PA7/808		10.81 z	
T85/799 X T65/238		10.46 α	
T63/967 X IMC60		10.30 β	
T63/967 X T17/524		9.71 γ	
	± S.E. 7.38	± S.E. 11.02	± S.E. 5.98

**Significant at P = 0.01, LSR value = 0.02172

Nitrogen content of cocoa leaves

Table 8 also showed the mean nitrogen content obtained from cocoa leaves through the kjeldahl method of extraction. Differences among treatments in terms of Nitrogen content were highly significant. PA150 x

NA33 had the highest Nitrogen content (2.244) in the leaves, and highly different from the others, while T85/799 x T65/238 had the lowest Nitrogen content of 1.502. T85/799 x Amelonado (1.976), T63/967 x IMC60 (1.969) Amelonado x Amelonado (1.957) were not different from each other. T63/967 XT17/524 (1.952) was also not different from T63/967 x IMC60 and Amelonado x Amelonado. T85/799 x T65/326(1.812) was different from T85/799 x PA7/808 (1.781).

T63/967 X T65/326 (1.683) was also not different from T85/799 x T79/501 (STANDARD) (1.670) but both were significantly different from T85/799 x T65/238(1.502)

Table 8 Mean nitrogen content (%/g dry matter) of cocoa leaves

Treatment	Nitrogen content
PA150 X NA33	2.244 a
T85/799 X AMEL (STANDARD)	1.976 b
T63/967 X IMC60	1.969 bc
AMEL X AMEL (STANDARD)	1.957 bc
T63/967 X T17/524	1.952 c
T85/799 X T65/326	1.812 d
T85/799 X PA7/808	1.781 e
T63/967 X T65/326	1.683 f
T85/799 X T79/501 (STANDARD)	1.670 f
T85/799 X T65/238	1.502 g

**Significant at P= 0.01 LSR value = 0.02175 ± S.E. 0.21

Means followed by a common letter are not significantly different from each other.

[illegible] (%/g dry matter) of cocoa type/ CSSV strain interaction

Cocoa Type	Healthy control	N1 strain	1A strain
T63/967 X T17/524	3.170 a		
PA150 X NA33	3.013 b		
T85 /799 X PA7/808	2.670 c		
Amelonado X Amelonado	2.650 c		
T63/967 X IMC660	2.613 d		
T85/799 X Amelonado	2.397 e		
T63/799 X T65/326	2.273 f		
PA150 X NA33			2.200 g
T85/799 X T65/238	2.100 h		
T85/799 X T65/326	2.090 h		
T85/799 X T79/501	1.980 i		
T85/799 X Amelonado			1.938 j
T63/967 X IMC60			1.800 k
Amelonado X Amelonado			1.790 k
T85/799 X T65/326			1.740 l
T85/799 X T79/501		1.610 m	
T85/799 X T65/326		1.607 m	
T85/799 X Amelonado		1.600 m	
T63/967 X T17/524			1.600 m
PA150 X NA33		1.520 n	
T63/967 X IMC60		1.493 o	

Table 9 Mean Nitrogen content (%/g dry matter) of cocoa type/ CSSV strain interaction (continued)

Cocoa Type	Healthy control	N1 strain	1A strain
T85 /799 X PA7/808		1.487 o	
Amelonado X Amelonado		1.430 p	
T85/799 X T79/501			1.420 p
T63/799 X T65/326			1.390 q
T63/799 X T65/326		1.387 q	
T85/799 X T65/238		1.307 r	
T85 /799 X PA7/808			1.187 s
T85/799 X T65/238			1.100 t
T63/967 X T17/524		1.087 t	
P = 0.01 LSR value = 0.02175	± S.E. 0.43	± S.E. 0.16	± S.E. 0.36

Table 9 showed the effect of the interaction between cocoa variety and Cocoa Swollen Shoot Virus strain in the production and utilisation of nitrogen in both infected and healthy cocoa varieties. From the table highly significant differences between the cocoa varieties in their response to Cocoa Swollen Shoot Virus attack. The healthy cocoa varieties produced more nitrogen than the infected cocoa types. Of the cocoa types T63/967 X T17/524 produced the highest quantity of 3.170 in healthy plant and closely followed by PA150 X NA33 with value of 3.013. PA150 X NA33 also produced a quantity of nitrogen in the 1A-infected tissues which was significantly greater than that

produced by T85/799 X T65/238 (2.100), T85/799 X T65/326 (2.090) and T85/799 X T79/501 (1.980).

Most of the cocoa varieties namely, T85/799 X Amelonado (1.930), T63/967 X IMC60, (1.800) and Amelonado X Amelonado (1.790) produced significantly higher levels of nitrogen than T85/799 X T79/501 (1.610), T85/799 X T65/326 (1.607) T85/799 X Amelonado (1.600) in the1A infected tissues than in the N1 infected tissues of the cocoa varieties.

Also T85/799 X T79/501 (1.420) T63/967 X T65/326 (1.390) produced more nitrogen than T63/967 X Y65/326 (1.387) and T85/799 X T65/238 (1.307) in the 1A infected tissues than the N1 infected tissues.

Molecular weight of total proteins of CSSV infected and healthy cocoa types

The molecular weights of proteins identified in swollen shoot virus infected and healthy cocoa type are presented in Table 10, and photographs of Plates 12 to 15 show the polyacrylamide gel electrophoresis of the resolved protein bands from which the molecular weights were calculated using the calibrated curves in Figs 9 to 12.

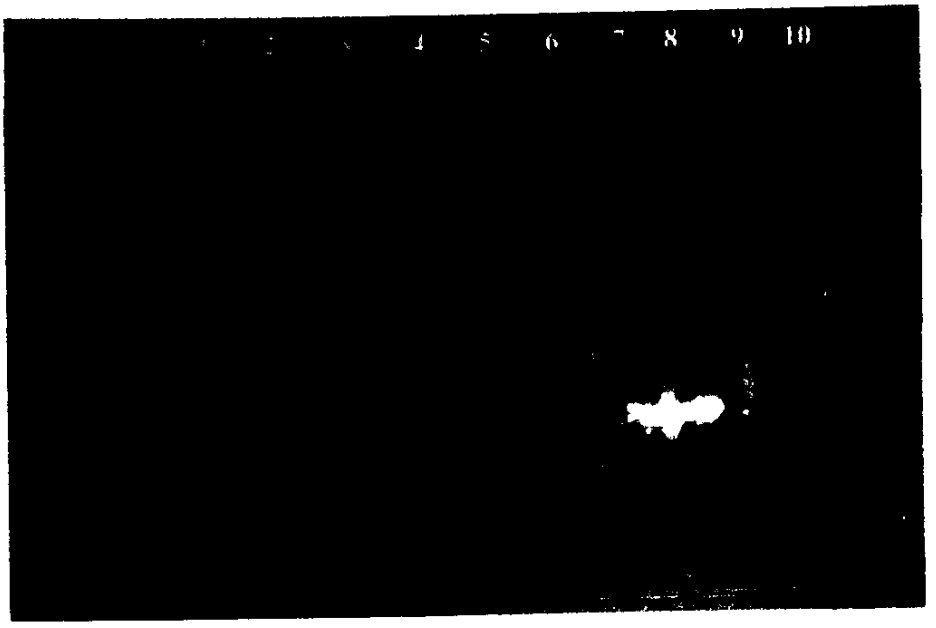

Plate 12(a): original gel loaded with samples of cocoa types in wells 2-10, and standard molecular weight marker in well (1). (x 2)

The gel plate 12(a) was loaded with samples of cocoa types in wells numbered 2-10. The standard molecular weight marker was loaded in the first cell. The order for loading is as follows: The healthy cocoa sample of the cocoa type (control) was always loaded first, followed by the sample infected with 1A strain of the CSSV and then the sample infected by the mild strain. Gel Plate "a" was loaded with samples from T85/799 X T65/238 in the second, third and fourth wells, T85/799 X T65/326 in the firth, sixth and seventh wells whereas T85/799 X PA7/808 was loaded in the eighth, ninth and tenth wells.

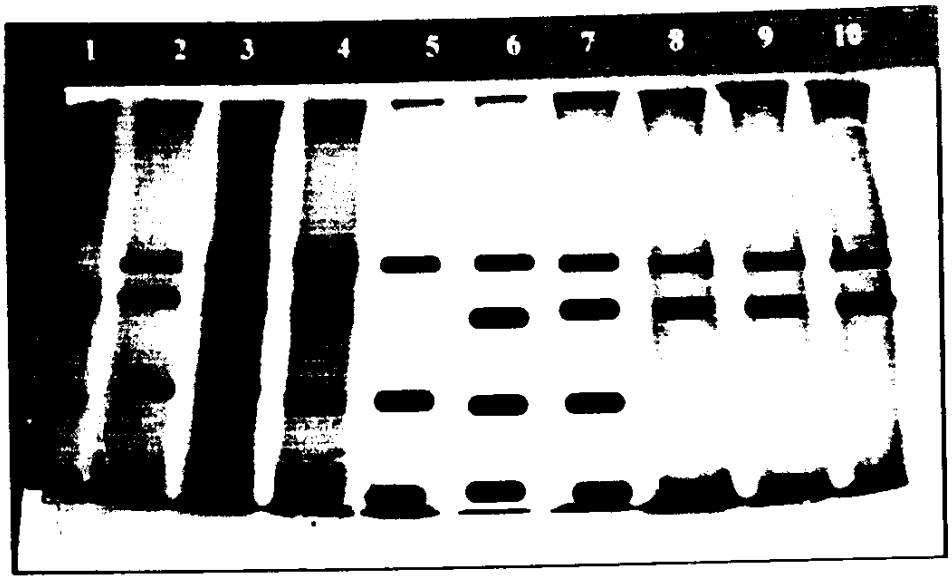

Plate 12(b): Gel taken from plate "a" and manipulated (x 2)

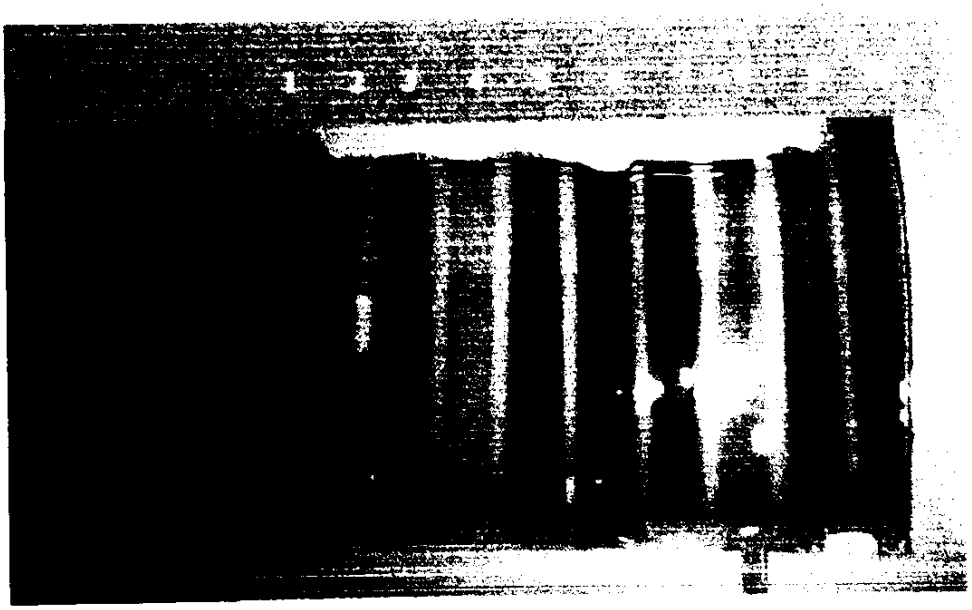

Plate 13 (a): Original gel taken from gel plate Number "b" (x 2)

Plate "b" (Plate 113a) was loaded with cocoa protein samples from T63/967 X IMC60 in second, third and fourth wells, T63/967 X T17/524 in the fifth, sixth and seventh well, and T63/967 X T65/326 in the eighth ninth and tenth wells.

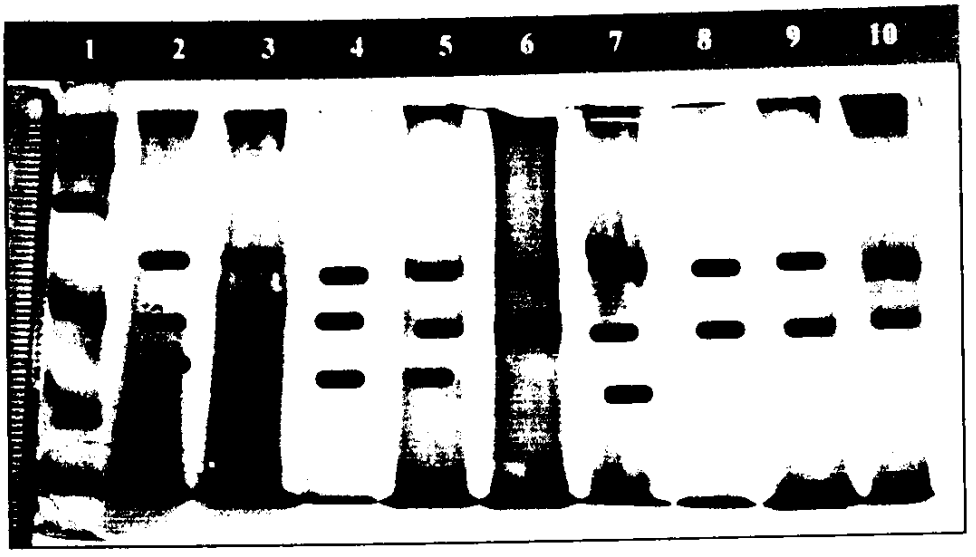

Plate 13 (b): Gel taken from Plate ('b') and manipulated (x 2)

Plate 14 (a): Original gel taken from Plate Number c (x 2)

Plate "c" (Plate 14 a) was loaded with samples from cocoa types namely, PA150 X NA33 in the second third and fourth wells whereas T85/799 X Amelonado in the firth sixth and seventh wells.

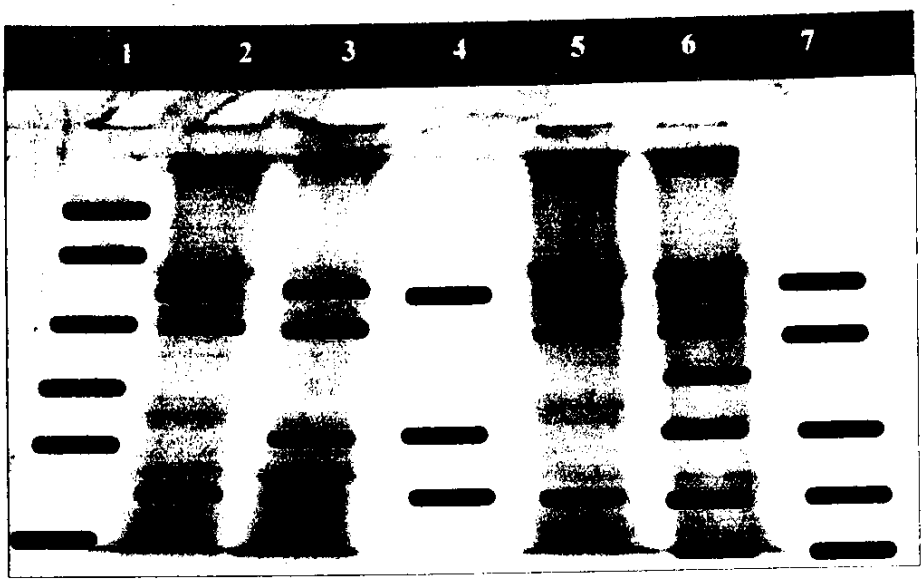

Plate 14 (b): Gel taken from Plate 'c' and manipulated (x 2)

Plate 15(a): Original gel taken from Plate'd' (x 2)

Gel plate "d" (Plate 15(a) was loaded with samples from the cocoa types Amelonado X Amelonado (standard) in well two; three and four whiles T85/799 X T79/501(Standard) in wells five, six and seven.

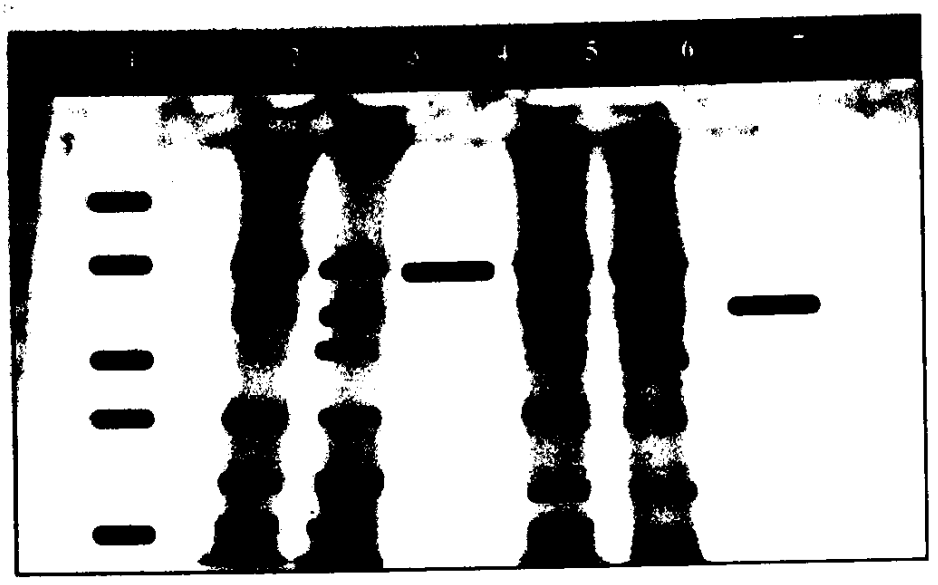

Plate 15(b): Gel taken from Plate "d" and manipulated. (x 2)

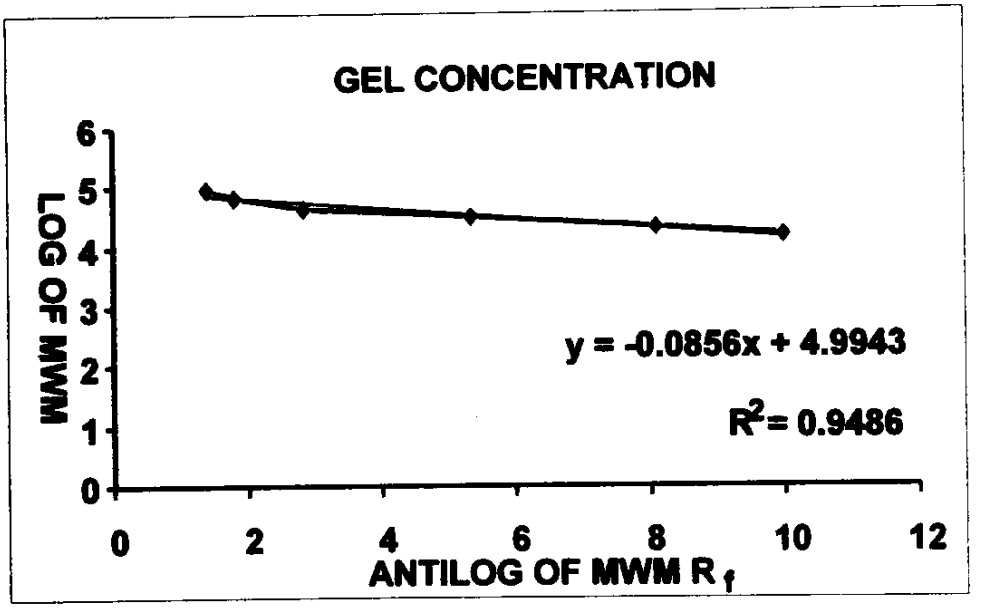

Fig 9 Calibration curve for Gel Plate A

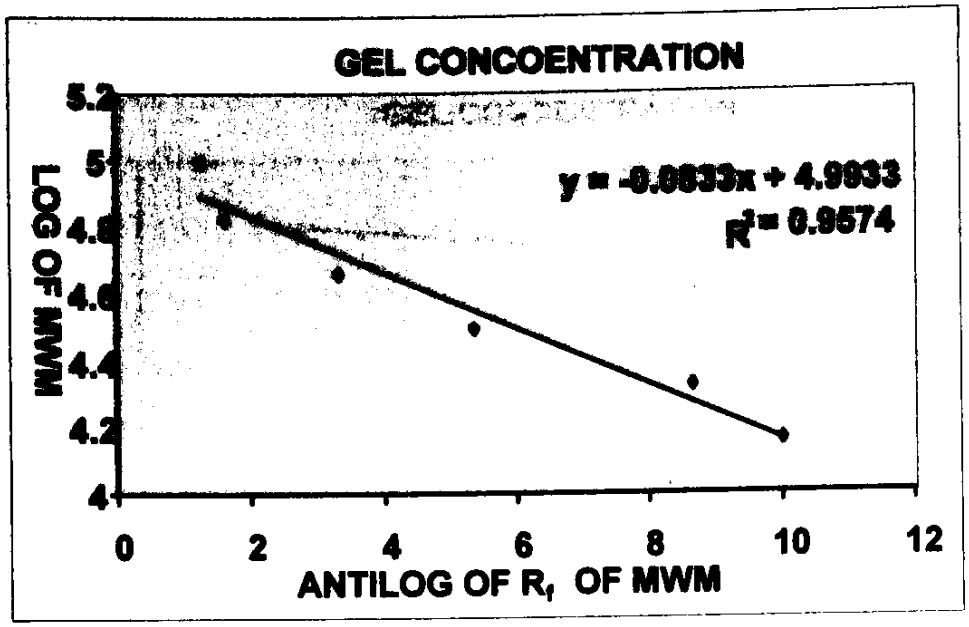

Fig 10 Calibration curve for Gel Plate B

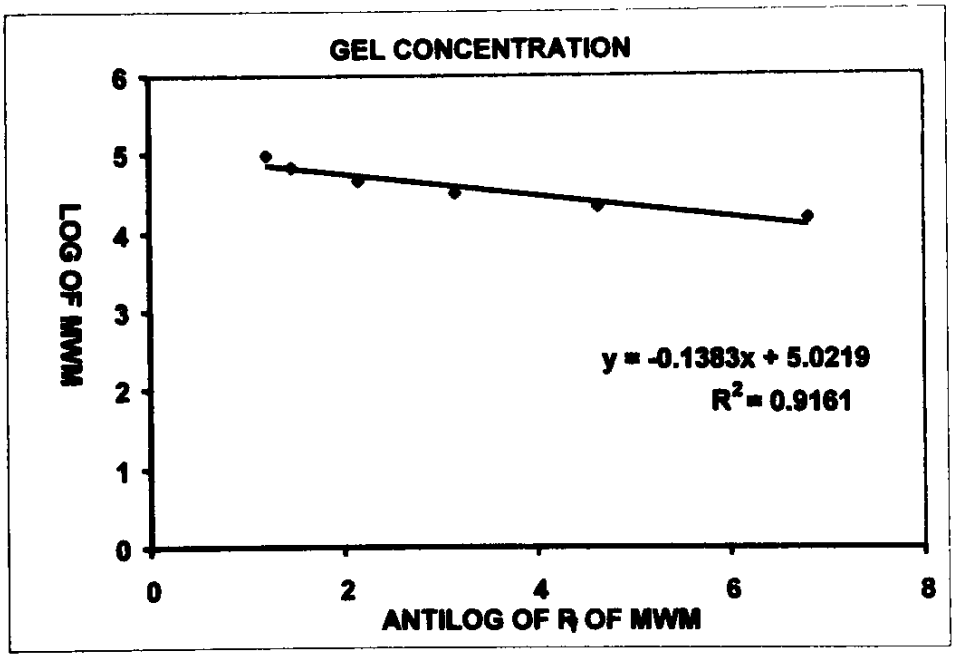

Fig 11 Calibration curve for Gel Plate C

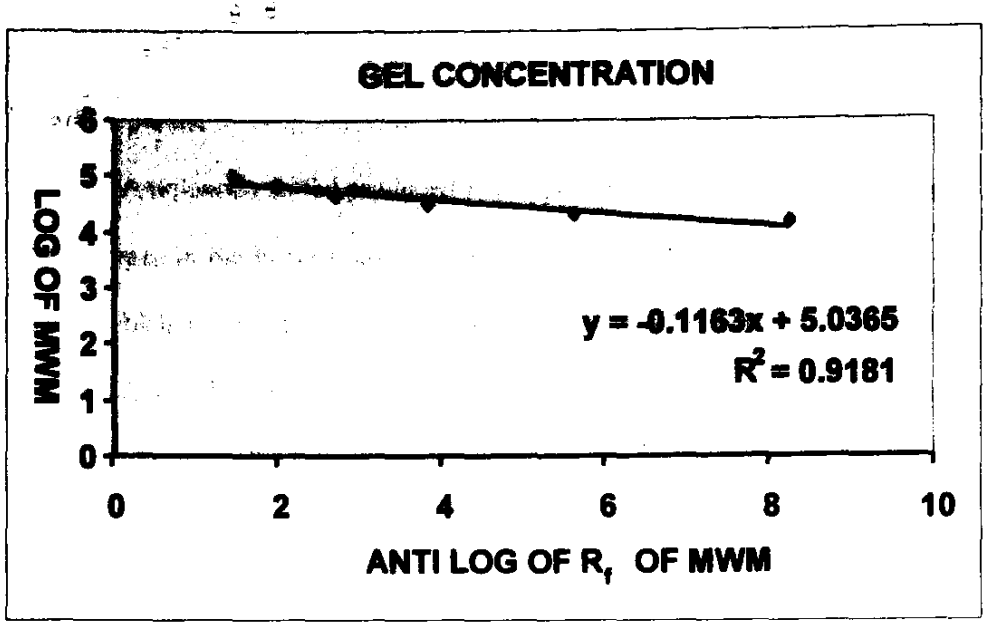

Fig12 Calibration curve for Gel Plate D

It can be seen from Table 10 that the molecular weights varied from 11.9 to 62.2 kDa. With the exception of T85/799 X Amelonado and T85/799 X T79/501 crosses, all the other crosses with T85 produced the same types of proteins in both the infected and healthy crosses, with the protein sizes ranging from 19.6 to 62.6 kDa. Similarly, all crosses with T63/799 tend to produce proteins of the same molecular sizes, ranging from the lowest of 43.8 to 59.6 kDa. It is interesting to note that the infected and healthy PA150 X NA33 produced protein of molecular weights 59.6 and 59.2 kDa as T63/967 crosses. They also produced protein of size 23.9 kDa, which was not in the T63/967 crosses and in all the healthy tissues of the cocoa types.

In the case of T85/799 X Amelonado, even though both the infected and the healthy produced two types of proteins of similar sizes that is 59.6 and 52.9 kDa, the CSSV 1A produced protein size 38.4 kDa in addition to the 23.9 kDa protein produced by the N1 mild strain which were not in the healthy

ones. Also, unlike PA150 X NA33, T85/799 X Amelonado produced proteins of lower molecular weights of 17.5 kDa and12.0 kDa in both infected and healthy plants.

Amelonado selfed produced four types of proteins in the CSSV 1A and three types in the healthy but only one type in the mild strain, which is 61.0 kDa, which is also found in both the healthy and the infected. The fourth protein (24.1 kDa) produced by Amelonado selfed was similar in size to 23.9 kDa produced by the infected T85/799 X Amelonado. It can be seen from the table that T85/799 X T79/501 produced four types of proteins with similar molecular weights in both infected and healthy cocoa tissues. These were 61.1, 52.6, 17.5 and 11.9 kDa. In addition, the CSSV infected produced two additional proteins of size 24.1 and 46.6 kDa. The mild strain infected tissues produced one type of protein of molecular weight, 61.0 kDa.

T85/799 X T65/238 produced the same types of proteins in healthy and infected tissues. These were 62.6, 49.4, 34.5, and 19.9 kDa. Apart from 19.9 kDa which was absent in the healthy tissues of T85/799 X T65/326, all the other proteins found in T85/799 X T65/238 were also found in T85/799 X T65/326.

T85/799 X PA7/808 produced proteins of weights 62.6, 49.4 and 19.9 kDa in both the healthy control and CSSV-1A infected tissues. Protein weight of 19.9 kDa was absent in mild N1 infected tissue. In T63/967 X IMC60, CSSV- 1A infected tissues produced 59.6 and 52.1 and 43.8 kDa proteins, whereas CSSV-N1 produced 59.6 and a different protein of 35.2 kDa. The healthy control also produced 59.6, 52.1 and 43.8 kDa.

There was no difference in the proteins produced by the CSSV-1A, and the healthy control in T63/967 X T17/524 as both produced protein of the weights 59.6, 52.1 and 43.8kDa. The mild strain (N1) Cocoa Swollen Virus infected tissues of T63/967 X T17/524 produced protein of weights 59.6 and 52.1 kDa. T63/967 X T65/326 produced the same types of proteins, 59.6 and 52.1 kDa, in healthy, CSSV 1A and N1 tissues.

Table 10 Molecular weight of proteins (kDa) of cocoa types

PROGENY	CSSV-1A	CSSV-N1	HEALTHY CONTROL
1. T 85/799 X T65/238,	62.6, 49.4, 3 4.5, 19.9	62.6, 49.4 34.5, 19.9	62.6 49.4, 34.5, 19.9
2. T85/799 X T65/326,	62.6, 49.4, 34.5, 19.9	62.6, 49.4 34.5, 19.9	62.6, 49.4, 34.5
3. T85/799 X PA7/808,	62.6, 49.4, 19.9	62.6, 49.4	62.6, 49.4, 19.9
4. T63/967 X IMC60;	59.6, 52.1, 43.8	59.6, 35.2	59.6, 52.1, 43.8
5. T63/967 X T17/524,	59.6, 52.1, 43.8	59.6, 52.1	59.6, 52.1, 43.8
6. T63/967 X T65/326,	59.6, 52.1	59.6, 52.1	59.6, 52.1
7. PA150 X NA33,	59.6, 52.9, 23.9, 17.5, 12.0	59.6, 23.9, 17.5	59.6, 52.9, 17.5, 12.0
8. T85/799 X AMEL	59.6, 52.9, 38.4, 23.9, 17.5, 12.0	59.6, 52.9, 23.9, 17.5, 12.0	59.6, 52.9 17.5, 12.0
9. AMEL X AMEL	61.0, 52.6, 38.9, 24.1	61.0	61.0, 52.6, 38.9
10. T85/799 x T79/501	61.0, 52.6, 46.6, 24.1, 17.5, 11.9	61.0	61.0, 52.6, 17.5, 11.9

CHAPTER FIVE

DISCUSSION

The observations from both gauze house and laboratory studies clearly indicated that all the cocoa types inoculated with Cocoa Swollen Shoot Virus 1A strain were susceptible to some extent to the cocoa virus by showing varied degree of severity in the symptoms they expressed.

The effects of the 1A virus were apparent within a month of infection and planting (Goodall, 1949).This suggested that the CSSV 1A virus was virulent and infective to all the cocoa types as reported earlier by Posnette and Todd (1951).

However, of the ten cocoa types used, PA150 X NA33, was the least affected by the virus in terms of symptoms expressed probably because it had an upper Amazon parentage introduce by Pound (1943) and more difficult to infect with the Cocoa Swollen Shoot Virus than the local Amelonado(Posnette and Todd,1951).T85/799 X T65/238 had the highest level of infection contrary to the observation made by Kenten (1975), that the parentage T85/799 and T65/238 were the least infected by Cocoa Swollen Shoot Virus than T63/967 and T79/501. There were no differences between next two susceptible cocoa types, T85/799 X Amelonado and T85/799 X T79/501. This may have resulted because both are series II hybrids and may have evolved from the same parentage.

The reference to Amelonado as a highly susceptible species by Posnette (1947) was supported by the findings of the CSSV screening in the gauze house as Amelonado X Amelonado was observed to have a significantly higher level of infection than the rest of the other cocoa types except T85/799 X T65/238. T85/799 X T65/326, T85/799 X Amelonado and T85/799 X T79/501 were not different from one another in terms of CSSV 1A infection. It could be that they are from the same genotype source (Abdul-Karim *et al.*, 2004).

The N1 mild strain did not show symptoms on any of the cocoa types, as did the severe New Juaben 1A strain confirming observations of Attafuah and Dale (1958) that the New Juaben isolate is the most virulent of Cocoa Swollen Shoot Viruses. The disease severity was higher on patch grafted seedlings than on those inoculated by mealy bug as a result of the inoculum pressure being too high in patch grafting than in mealy bug transmission of the cocoa swollen shoot virus as observed by Adu- Ampomah *et al.*, (2002).

It was noted that, the total polyphenol content in cocoa types was influenced by viral infection. The amount of total polyphenols content increased with infection with the severe 1A viral strain as reported by Kumar, (1991), Sharma and Chowfla, (1991), and Suresh *et al.*, (1991). However, the amount of the total polyphenols in N1 mild strain infected tissues was significantly lower than the amounts found in healthy plant tissues probably because the N1 mild strain was not potent enough to influence the cocoa types to induce the production of polyphenols. It could also be that the total polyphenol contents produced by the cocoa types during infection with N1 mild strain virus were utilised to offset the effect of the virus.

There were significant differences between the levels of total polyphenolic content of the cocoa varieties. T85/799 X T79/501 had the highest content of 32.25 g/kg of total polyphenolics among the cocoa types used. It did not, however, have the lowest infection level as polyphenols have been implicated in providing immunity to plants (Harborne, 1995). It could be that not all polyphenols confer immunity to plants as observed by Nimal *et al.*, (2005). Constitutively, PA150 X NA33 and T85/799 X T79/501 had the lowest amounts of total polyphenols. These amounts were induced over 74 and 108 % after infection with CSSV 1A strain and 148 and 141 % respectively after infection with CSSV N1 mild strain, but Baruah and Chowfla, (1994) suggested that there were higher levels of polyphenols in healthy plants and these higher contents of secondary metabolites in healthy plants protected them from infection. The results of the study revealed that there is weak correlation (0.334) between total polyphenols content and susceptibility to CSSV infection in the cocoa types which somewhat confirmed an earlier findings by Adomako (1974) that there is no correlation between polyphenols content and susceptibly to CSSV.

The observation that nitrogen content decreases with infection in both 1A and N1 infected issues was at variance with the observations of Adomako and Hutcheon (1974) that no differences existed in the nitrogen levels of the infected and healthy cocoa tissues. In this study, the 1A-infected cocoa tissues however, produced more nitrogen than the N1 infected tissues. The nitrogen content of PA150 X NA33 was significantly higher than the rest of the cocoa types.

There was a strong correlation (-0.98) between nitrogen content and susceptibility to Cocoa Swollen Shoot Virus disease. PA130 X NA33 which had the highest nitrogen content was the least infected by the virus.

A simple but efficient method of resolution of total proteins from infected and healthy cocoa tissues was used to resolve many different proteins. The protein profiles detected were found to be akin to the pathogen.

The presence of some proteins in certain cocoa types and the absence in others suggested that such proteins may be pathogenic. Those thought to be pathogenic related proteins compared favourably in size with Cocoa Swollen Shoot Virus and other badnaviruses (Dzahini-Obiatey *et al.*, 2002). For example 23.9/24.1 and 46.6 kDa proteins observed in this study are close in size to the 22.4, and 46.0 kDa proteins obtained by Hughes *et al.*, (1994, 1995) The 23.9 kDa protein is also close in size to the 23.0 kDa putative protein of the open reading frame 1 (ORF1) of the sequence data of the Commelina Yellow Mottle Virus (Medbery *et al.*, 1990).

The close relationship between the 23.9 kDa protein and one of the severest isolates, (CSSV 1A) suggested the functional role of the protein (Dzahini-Obiatey *et al.*, 2002). The presence or absence of certain resolved protein weights of tissues of the cocoa types may give an indication whether the plant is attacked by the 1A isolate or the N1 isolate.

The results on molecular weights of proteins revealed the synthesis of many proteins, some of which may be pathogenic related. A protein weight of 23.9 kDa was resolved in PA150 X NA33 in the CSS1A, and N1 tissues. This same protein weight was found in the CSSV infected tissues of T85/799 X T79/501 and Amelonado X Amelonado and both CSSV1A and N1 tissues of

T85/799 X Amelonado. The absence of 23.9 kDa protein in the healthy tissues suggested that the 23.9 kDa protein may be pathogenic. The functional role of this pathogenic related protein (Dzahini-Obiatey *et al.*, 2002) may be in the disease initiation in the cocoa types found. The presence of 23.9 kDa protein size may be responsible for the significantly high incidence of CSSV observed on PA150 X NA33. However, the significant amount of nitrogen content in PA150 X NA33 may have offset the severe effect of the pathogen. Similarly, the low level of nitrogen coupled with the presence of protein weight of 24 kDa may have been responsible for the high susceptibility of Amelonado X Amelonado. The combined effects of 46.6 kDa and 24.1 kDa protein besides the low amount of nitrogen in T85/799 X T79/501 may have accounted for its susceptibility to the Cocoa Swollen Shoot Virus.

The proteins with approximate size of 62.6, 61.0, 59.6 kDa, and 52.1 and 49.4 kDa present in both the healthy and CSSV 1A infected tissues could therefore not be associated with CSSV infection (Dzahini-Obiaatey *et al.*, 2002). It is also not very certain the role played by the other proteins size such as 38.4, 34.5, 19.9, 17.5 and 12.0 kDa. They may be present initially in the tissues but with time may disappear in the healthy tissues and become pathogenic related may be from the plant host origin rather than due to CSSV infection.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

From the study the findings are summarized as:

I. There were different levels of resistance of cocoa types to the Cocoa Swollen Shoot Virus.

2. All the cocoa types screened were susceptible to the Cocoa Swollen Shoot Virus 1 A strain and not the mild strain.

3. The CSSV reduced the level of nitrogen in infected cocoa types.

4. There is a strong negative correlation between nitrogen content and CSSV disease resistance of cocoa types.

5. Graft inoculated cocoa types succumbed to the effects of the virus than the mealy bug inoculated cocoa types.

6. PA150 X NA 33 was found to be a promising cocoa variety for breeding purposes.

7. Amelonado is still a very susceptible cocoa type.

8. Proteins, rather than total polyphenols were responsible for resistance in cocoa.

9. Proteins of approximate molecular weight of 23.9 kDa and 46.6 kDa may be responsible for Cocoa Swollen Shoot Virus disease initiation and progression.

10. Total polyphenols increased with CSSV 1A strain infection and decreased with the N I mild strain infection.

11. [illegible] were least affected by the Cocoa Swollen Shoot Virus.

12. Nitrogen content and molecular weight of proteins can be used to assess CSSV disease resistance.

Conclusion

The studies revealed that biochemical changes occurred in Cocoa Swollen Shoot Virus infected cocoa plants. However, the occurrence of some pathogenic proteins in some cocoa types infected with CSSV is indicative of the possibility of the considerable variation in resistance levels. These variations in the resistance levels of the promising varieties could be utilized in the development of new cocoa varieties with predictable level of CSSV resistance.

Recommendations

On the basis of the findings of this study, it is recommended that:

I. The screening of cocoa types through biochemical means should be repeated.

2. High Powered Liquid Chromatography (HPLC) should also be used to determine quantities of the various phenolics in the cocoa types and relate them to CSSV infection.

3. Further work should be done to monitor the levels of total polyphenols and nitrogen as well as the molecular weights of proteins, protein synthesised and their utilization at intervals during growth of the cocoa types.

4. Further breeding work should be done to increase nitrogen levels of the cocoa types constitutively.

5. Amelonado can still be used as a susceptible test plant.

6. Crosses between PA150 X NA33 and T63/967 X T17/534, and between PA150 X NA33 and T63/967 X IMC60 could be done to obtain other hybrids for laboratory analysis because they appeared to be promising resistant varieties.

REFERENCES

Abdul Karim, A. Adomako, B. and Adu-Ampomah, Y. (2004). Cocoa introduction into Ghana. *Ghana Journal of Agricultural Science*, 38(2) (in press)

Ackonor, J. B. (I 977). Preliminary studies on breeding and predation on *Scymnus (Pullus)* sp. and *Hyperaspis egregia* Mader on *Planococcoides njalensis* Laing: *Proc. 1st Cocoa Pests and Diseases Seminar. Accra, Ghana. 6-10 November, 1995*: 238-241.

Adegbola, M.O.K. (1971). Significant developments in the study of the Cocoa Swollen Shoot Virus disease. *Bulletin of Entomological Society of Nigeria* 3: 6–18.

Adegbola, M. O. K. (1973). Cross protection reactions of multiple strains of CSSV. *Ann.Rep. Cocoa Res. Inst. Ngr. Ibadan.* 1971/72: 150-152.

Adejumo, T.O. (2004). Crop Protection Strategies for major diseases of cocoa coffee and cashew in Nigeria. *African Journal of Biotechnology* vol.4 (2). February 2005. pp. 143- 150.

Adenikinju, S.A; Esan. E.B: Adeyimi, A.A. (1989). Nursery techniques, propagation and management of cacao, kola, coffee, cashew and tea. Pp.1-27. In: *Progress in Tree Crop Research* (2nd Edition), Cocoa Res. Inst. Ngr. Ibadan.

Adeyimi, A. A. (1996). NPK fertilizer in rehabilitated cocoa: Effects on the growth and development of cocoa under different methods. *Proc. 12th Int. Cocoa Res. Conf Salvador, Bahia, Brazil,* Nov1996. (CNC) from cocoa leaves. *Rep Cocoa Res Inst. Ghana.*

Adomako, D. (1974). Biochemical studies on varietal resistance of cocoa to CSSV. *Cocoa Research Institute Annual Report* 1974-75

Adomako, B. (1977). Fatty acid composition and characteristics of *Pentaclethra butyracea* Fat extraction from Ghana seeds. *J.Sci. Fd. Agric.* 1977, 28, 384-386

Adomako, B. Allen, R.C. and Adu-Ampomah, Y. (1999a). Combining abilities for yield and vegetative trials of Upper Amazon cocoa selections in Ghana. *Plantations Recherché, Development* 6(3), 183 -189.

Adomako, B. Allen, R.C and Adu-Ampomah, Y (1999b). Evaluation of hybrids between Upper Amazon cocoa selections in Ghana. *Plantations Recherché Developpement* 6(6), 455-462.

Adomako, B. and Adu-Ampomah, Y. (2003). Bean characteristics of progenies of Upper Amazon cacao in Ghana. *Tropical Agriculture (Trinidad)*, 80 (1),41-47.

Adomako, D. Hutcheon W. V. (1974). Carbohydrates Metabolism and Translocation in Healthy and CSSV - Infected Cocoa Plants. *Physiol. Plant* 30:90 – 96

Adu-Ampomah, Y. (1996). The cocoa breeding program in Ghana: Achievements and prospects for the future. *Cocoa Growers' Bulletin* 50: 17-21.

Adu-Ampomah. Y. Adomako, B. Owusu. G.K. and Ollennu, L.A.A. (1996) Breeding for resistance to the cocoa swollen shoot virus in Ghana. I. *Proc. International Workshop on the contribution of disease resistance to cocoa variety improvement.* (INGENIC).24-26 November 1996, Salvador, Bahia, Brazil, 173-179.

Adu-Ampomah, Y. Ollennu, L.AA, Adomako, B. and Padi, B. (2002).

Breeding nd genetics of Cocoa Swollen Shoot Virus Disease Plant Viruses Online: Descriptions and Lists from the VIDE Database. Resistance in *Theohroma cacao* L. *Journal of the Ghana Science Association* vol. V no. 2 pp 10-23.

Adu-Ampomah, Y. Ollennu, L.A.A. and Adomako, B. (2004). Evaluation of resistance to Cocoa Swollen Shoot Virus (CSSV). Method, problems and solutions. In: *Proceedings. Int. Workshop on Cocoa Germplasm Evaluation and Improvement through Global and participatory Approaches,* 28th March-3rd April, 2004(in press).

Andrews, A. T. (1986). Electrophoresis: Theory, techniques, and biochemical and clinical application, 2nd ed., Clarendon Press, Oxford,

Anon (1951). Capsid Research: Chemical control. *Ann. Rep. W. Aft Cocoa Res. Inst.* 1949-50: 40-42.

Anon (1990). Analysis of constraints on adoption of CRIG recommendations in Offinso District: Results and discussion. (C.P. Henderson and A. P. Jones ed.): 15pp. Cocoa Research Institute, Tafo, Ghana.

Anon (1995). *Report on causes of recent decline in cocoa production in Ghana and measures to revamp the industry.* Office of the President, Accra: 103 pp.

Armstrong, K. B. (1961). Systematic insecticides. *Report of West African CocoaResearch Institute.* 1959-60

Attafuah, A. Blencowe, J.W. and Brunt, A.A. (1963). Swollen Shoot Disease of cocoa in Sierra Leone, *Trop. Agric. Trin.* 40: 229

Attafuah, A. and Dale, W.T. (1957). Classification and comparison of

Mayo. Isolation, Serology of isolates and their effect on growth. *Annual Report of West African Cocoa Research Institute.* 1956-57, page 24-26.

Attafuah, A. and Glendinning, D.R. (1965a). Studies on resistance and tolerance to cocoa viruses in Ghana. I. A survey of TI 7 progeny. *Annals of Applied Biology* 56:219.

Attafuah A. and. Gladdening, D.R. (1965b) Studies on resistance and tolerance to cocoa viruses in Ghana. I. A survey of breeding material. *Annals of Applied Biology* 56: 227.

Baruah, B.P. and Chowtla, S.C. (1994). Physico-chemical changes in healthy and Turnip mosaic virus-ordinary strains infected cauliflower. *Indian J. Hill Farm*, 7, I: 57-61.

Bateman, M.J. (1988). Ghana Cocoa Pricing Policy study. *Commodity information Inc.* 1981: 77pp.

Bigger, M. (1981). The relative abundance of mealy bug vectors of Cocoa Swollen Shoot Disease in Ghana. *Bull. Ent. Res.* 1:435-448.

Bio-Rad Laboratories,(1987). Acrylamide polymerization- A practical approach. *Bulletin* 1156

Blencowe, J.W. Brunt, A.A. Kenten, R.H. and Lovi. N.K. (1963). A new virus disease of cocoa in Sierra Leone. *Tropical Agriculture Trinidad* 40: *233-23h.* Blencowe, J. W. (1971). Cocoa and coconuts in Malaysia. *The Planter* 47:47 299-303.

Blum, H. Beier, H. Gross, H.J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis.* 8:93-99.

Borgo, M. (1991). Influenza delle viroси dell'accartocciamento fogliare della vite su alcuni parametri della produzione. *Riv. Vitic. Enol.*, 44, 2: 21-30.

Boss, L. (1978). *Symptoms of Virus Diseases in Plants*. Pudoc Wageannigen.

Bowers, J. H. Bailey, B.A. Hebbar, P. K. Sanogo, S. and Lumsden, R. D. (2001). The impact of plant diseases on World Chocolate production.. Online. Plant Health Progress doi: 2001 (Peer-Reviewed). 10.1 094/PHP 2001-0709-01-RV.

Broadbent, L. (1964). Control of plant virus disease. In *Plant virology* Corbett and Sisler (Eds) Univ. Fla. Press, Gainsville. p527.

Brown J.R, (1975). Fed. *Proc. Fed Am Soc. Exp. Biol*, 34,591

Brunt, A.A. (1970). Cocoa Yellow Mosaic Virus. CMIIAAB Descriptions of Plant Viruses no. II. Commonwealth Mycological Institute, Kew. UK

Brunt, A.A. (1986). Cocoa Swollen Shoot Badnaviruses. Viruses of plants. *Description and list from the V/DE Database* pp.278-280

Brunt, A.A. and Kenten, R.H.(1962). The mechanical transmission of Cocoa Swollen Shoot Virus to and from cocoa and other hosts. *Annals of Applied Biology* 50, 749-754.

Brunt, A.A and Kenten, R. H. and Nixon, H.L. (1964). Some properties of Cocoa Swollen Shoot Virus. *Journal of General Microbiology* 36: 303-309

Brunt, A.A and Kenten, R. H. (1971). Viruses infecting cacao. *Review of Plant Pathology* 50: 591-602.

Brunt, A.A, Crabtree, K. Dallwitz, M.J. Gibbs, A.J. Watson, L. and Zurcher,

[illegible] *Descriptions and Lists from the VIDE Database*. CABI.

Brunt, A.A. Crabtree, K. Dallwitz, M.J. Gibbs, A.J. Watson, L. and Zurcher, E.J. (eds.) (1996). Plant Viruses: Online: *Descriptions and Lists from the VIDE Database*. Version: 20th August I. I URLhttp: //biology anu. edu. au/Groups/MES/vide/

Campbell, C.A.M. (1983). The assessment of mealy bugs (Pseudococcidae) and other Homoptera on mature cocoa trees in Ghana. *Bull. Ent. Res.*, 73:137-151

Carter. W. (1956). Notes on some mealy bugs (Coccidea) of economic importance in Ceylon. *FAO Plant Bulletin* 4: 49.

Cornwell, P.B. (1960). Movement of' the vectors of virus diseases of 'cocoa in Ghana.II; Wind movement and aerial dispersal. *Bulletin of Entomological Research* 5I. 175-20 I

Dale, W.T. (1957). Studies on resistance and tolerance to cacao viruses. *Cocoa Breeding Conference, Ghana.* Page 3.

Dale, W.T. (1962). Virus diseases. *Agriculture and land use in Ghana.* Oxford University Press. UK Pages 286-316

Davis, R.P. (1971). "Carbonic Anhydrase." in: *The Enzymes*, vo1.V 545, (Boyer, P.D. Ed.) Academic Press, New York

Decker, F. E. (I 955). Mealy bug studies; biological control of mealy bugs. *Annual Report West Africa Cocoa Research Institute*, 1953, p23.

Donald, R.G. (1953). Mealy bug studies: Introduction of exotic parasites. *Annual Report West Africa Cocoa Research Institute*, 1953, p23.

Donald, R.G. (1955). Mealy bug studies. *Annual Report of West Africa Cocoa*

[redacted], 1998/54: 101

[redacted] Akuffo P.C. Takrama, J.F. Lowor, S.T. and Ollenu L.A.A. (2002). A method for the elucidation of pathogenic related proteins of Cocoa Swollen Shoot Badnavims. *Journal of the Ghana Science Association*, vol. 4 no.3 2002.

Esan, E.B. (1977). Tissue culture studies in Cacao (*Theobroma cacao* L) A Supplementation of current research. In: *Proceedings of the 5th International Cocoa Research Conference*, 1975. Cocoa Res. Inst. Nigr. Ibadan 116-125.

Esan, E.B. (1982). Root regeneration from callus derived from embryo axis cultures of *Theobroma cacao in vitro. Turrialba* 2: 3359-364.

Feucht, W. (1994). The localization of phenols at the cellular and tissue level. *Acta hortic* 381: 803-815

Firempong, S. (1984). Screening of insecticides for mealy bug control *Rep. Cocoa Res. Inst., Ghana*, 1976/77-1978/79: 72.

Gill, and Duffus, (1989). Cocoa Statistics. *Gill and Duffus Gp.* Ltd.: 29 pp.

Glendinning, D.K (1964) A study of clonal cocoa varieties. *Horticultural Research* 4(2), 89-97.

Glendinning, D.K. (1967). New Cocoa varieties in Ghana. *Cocoa Growers Bulletin* 8, 19-24.

Goodall, D.W. (1949). Virus disease of Cocoa in West Africa IV. Effect of virus infection on growth and water content of Cacao seedlings. *Annals of Applied. Biology*.36, 440-447.

Gottlieb, O. R. (1990). Phytochemicals: differentiation and function. *Phytochemistry* 29, pp. 1715-1 724

Guidoni, S., Mannini, F., Fumagalli, A., Argamante, N. and Di Stefano, R.. (1997). The effect of Grapevine leafroll and Rugose wood sanitation on agronomic performance and berry and leaf phenolic content of Nebbiolo clone *(Vitis vinifera* L.). *Am. J. Enol. Vitic.*, 48, 4: 438-442.

Guttmann, M. and Feucht, W. (1994). Histochemical localization of catechins and procyanidins. *Acta hortic.*, 381 : 797-801

Hall, W.G. (1945). The identity of mealy bug vector of Swollen Shoot Virus disease of cocoa in West Africa. *Bul. Ent Res.* 36: 305-313.

Hannah, A. D. and Heattherington, W. (1957). Arrest of the Swollen Shoot Virus disease of cocoa in the Gold Coast by controlling the mealy bug vectors with the systemic insecticide, Dimefox. *Ann. Appl .Bio/.*, 45: 473-480.

Harborne, J. B. (1995). Plant polyphenols and their role in plant defence mechanisms. V: *Polyphenols* 94, Palma de Mallorca (Spain), May 23-27, 1994. Paris. INRA: 19-26.

Holden, M. (1957). An investigation on polyphenolic compounds of cocoa leaf in connection with a chemical method for detecting virus infection - *J. Sci. Food Agric.* 8: 553 - 561.

Hughes, J. D'A. Sackey, S.T. and Owusu, G.K. (1994). CSSV pathogenicity related nucleic acids and proteins. *Rep. Cocoa Res. Inst.*, Ghana, 1991/1992 pp.130-133.

Hughes, J. D'A. Adomako, D. and Ollennu, L.A.A. (I995). Evidence from the virobacterial agglutination test for the existence of eight serogroups of Cocoa Swollen Shoot Virus. *Annals of Applied Biology* 1227, 297-307

Hutcheon, W. V. (1973) Studies on the physiology of plants infected with

Trinidad and Tobago, Jan. 1972.

Jollès, P. and Angew, (1969). Lysozymes hydrolysis β(1→ 4) Linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine. *Chem. Int. Ed.* 8, 222-2.

Kaur, G. Gill, C.K., Rataul, H.S. and Raheja, R.K. (1991). Biochemical changes in Soya bean (*Glycine max*) L.O cuJtivars infected with Yellow Mosaic Virus. *Biochem. Physiol. Pflanzen* 187:357-371.

Kaur, G. Raheja, R .K. Ahuja, K.L. Singh, M. and Labana, K.S. (1989). PhysicochemicaJ changes associated with Peanut clump virus in *Arachis hypogaea* L. *Biochem. Physiol. Pflanzen*, 185: 209-276.

Kennedy, A.J. Lockwood, G.I. Mossu, G. Simmonds, N.W. and Tan, G.Y. (1987). Cocoa breeding: past, present and future. *Cocoa Growers Bulletin* 38: 5-22.

Kenten R.H. (1975). Resistance ranking on first flush infection. *Cocoa Research Institute Annual report* 1974-75

Kenten, R.H. and Legg, J.T. (1965). Observations of the purification and properties of Cocoa Swollen Shoot Virus. *Ghana J. Sci.* 5:221

Kenten, R.H. and Legg, J.T. (1970). Methods for assessing the tolerance and resistance of different types of Cocoa Swollen Shoot virus. *Annals of Applied Biology* 65:419-424.

Killick (1961) A study of the Contemporary Ghana, volume one- The economy of Ghana. Edited by W. Bermingham, I. Neustdt and E.N. Omaboe. London: Allen and Unwin, 1966.

Knight, R.C. and Rogers, H.H. (1955). Recent introductions to West Africa of

Lester, R.N. and Thresh, J.M. (1966). *Theobroma cacao* and related species. *Empire Journal of experimental Agriculture*. 23 (9), 113-125.

Knight, R.C. and Tinsley, T.W. (1958). Some Histological Observations on virus-infected *Theobroma* cacao L *Ann. Appl. Biol.* 46:7-10

Kumar N.N.U. (1991). Effect of Mosaic Virus on physiology of mulberry (*Morus alba* L.) *Ad. Plant Sci.*, 4, 1: 183-185

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685

Legg, J.T. and Agbodjan, (1969).Host range of Cocoa Swollen Shoot Virus. *Rep. Cocoa Res. Inst. Ghana.* 1967-68:25.

Legg J. T. and Kenten, R.H. (1968). Some observations on cocoa trees tolerant to Cocoa Swollen Shoot Virus. *Tropical Agriculture Trinidad* 45:61

Legg, J.T. and Lockwood, G. (1977). Tolerance and resistance to Cocoa Swollen shoot virus. In: *Proceedings, 5th International Cocoa Research Conference, Ibadan. Nigeria,* pp.403-408. Coca Research Institute, Ibadan.

Legg, J.T. Lockwood, G. and Bonney, J.K. (1972). Cocoa Swollen Shoot Virus. The screening of progenies in seedling tests. *Crop Research Institute Report* (1971-72)

Lister, R. M. (1953) Virus research In: *Annual Report of the West African Cocoa Research Institute.* 1952/1953. Pg. 9.

Lockard, R.G. and Asomaning, E.J.A. (1965). Mineral Nutrition of Cacao. II Effects of Swollen Shoot Virus on the growth and nutrient content of plants grown under nutrient deficient, excess and control conditions in sand culture. *Tropical Agriculture Trinidad* 42,39-53.

Lockhart, B.E.L., Olszewski, E.N. and Hull, R. (1995). Genus Babnavirus. In Virus Taxonomy, Sixth report of the international committee on taxonomy of viruses, Morphy , (eds) Arch V, vol8 supple.10 185-188.

Longworth, J.F. and Thresh, J.M. (1963). Field trial on the effect of a Nigerian Swollen Shoot Virus on the growth of different cocoa types. *Annals of Applied Biology* 52:217.

Lot, H. Kjiekpor, E. and Jacquemond, M. (1991). Characterization of the genome of Cacao Swollen Shoot Virus. *J Gen Virol* 72:1735-1739.

Mapother, H.R. and Nicol, J. (1953). Mealy bug studies. *Rep. W. Afr. Cocoa Res. Inst.*, 1950-51:40.

Marchart, H. (1963). Uptake of Systemic Insecticides. *Rep. Cocoa Res. Inst. Ghana*, 1967/68:40.

Medberry, S.L.Lockhart, B.E.L. and Olszewski, N.E. (1990). Properties of Commelina Yellow Mottle Virus's complete DNA sequence, genomic discontinuities and transcripts suggest that it is a pararetrovirus. *Nucleic Acid Research.* 18, 5505-5523.

Nimal Punyasiri, P.A. Sarath Abeysinghe, B. I. and Vijaya Kumar, (2005). Performed and Induced Chemical Resistance of Tea Leaf against *Exobasidium vexans* Infection. *Journal of Chemical Ecology* Publisher: Springer Netherlands ISSN: 0098-00331 (paper) 1573-1561 (Online) DOI: 10.1007/s10886-005-5288-z Issue: Volume 31, Number of Pages: 1315-1324

Ollennu, L.A.A. Owusu, G.K. and Thresh, J.M. (1989). The control of cocoa swollen shoot disease in Ghana. *Cocoa Growers' Bulletin* 42:25-35.

Owusu, G.K. (1971). Cocoa Necrosis Virus in Ghana. *Tropical Agriculture,*

[redacted]

Owusu, G.K. and Kenten, R.H. (1972). Cocoa Necrosis Virus. *Rep. Cocoa Res.Inst. Ghana.* 1969-70: 61.

Padi, B. (1997). Prospects for the control of cocoa mealy bugs. *Proc. 1st Int. Cocoa Pests and Diseases Seminar.* 6-10 Nov, 1995, Accra, Ghana: 249-263

Padwick G.W. (1956). Losses caused by plant diseases in the colonies. *Phytopathol.* Papers 1, 1-60.

Paulin, D. and Eskes, A.B. (1995). Cocoa: Breeding strategies. *Plantation Recherché, Developpement* 5(6), 5-18.

Pereira, J.L. (1996) Prospect for effective control of cocoa diseases. In: Proc. *12th International cocoa Research Conference, Salvador, Brazil,* November 17-23, 1996, Pp. 81-86.

Porter, L.J. MA, Z. and Chan, B.G. (1991). Cacao procyanidins: Major Flavanoids and identification of some minor metabolites. *Phytochemistry*, v.30, n. 5, 1657-1663.

Posnette, A.F. (1940). Transmission of swollen shoot diseases of cocoa. *Tropical Agriculture Trinidad* 17: 98.

Posnette, A.F. (1941) Swollen Shoot Virus disease of cacao. *Tropical Agriculture. Trinidad.* 18, 87.

Posnette, A.F. (1944). Vector investigations. *Quarterly Report of the West African Cocoa Research Institute* (Mimeographed)

Posnette, A.F. (1947). Viruses of cocoa in West Africa: 1 Cocoa viruses 1A, 1B, 1C and 1D. *Annals of Applied Biology* 34:388-402.

Posnette, A.F. (1950). Virus disease of cocoa in West Africa VII. Virus

transmission by different vector species. *Annals of Applied Biology* 37:378.

Posnette, A.F. (1951). Virus research at the West African Cocoa Research Institute, Tafo Gold Coast. *Tropical Agriculture, Trinidad* 28:133-142

Posnette, A.F. (1981). Viruses and resistance to virus diseases in cocoa. *Proceedings 6th International Cocoa Research Conference, Caracas, Venezuela*, 177, pp. 262-280.

Posnette, A.F. and Robertson, N.F. (1950).Virus Disease of Cocoa in West Africa VI: Vector Investigation. *Annals of Applied Biology*. 37:363-377.

Posnette, A.F. Robertson, N.F. and. Todd, J.M (1950). Virus diseases of cocoa in West Africa. V: Alternative host plants. *Annals of Applied Biology*. 37:229-240.

Posnette, A.F. and Strickland, A.H. (1948). Virus diseases of cocoa in West Africa. Technique of insect transmission. *Annals of Applied Biology* 35:53-63.

Posnette, A.F. and Todd, J.M. (1951). Virus diseases of cocoa in West Africa. VIII.The search for virus-resistant cacao. *Annals of Applied Biology* 38:785-800

Posnette, A.F. and Todd, J.M. (1955). Virus diseases of cocoa in West Africa. IX. Strain variation and interference in virus 1A. *Annals of Applied Biology* 43:433-453.

Pound, F.J. (1943). Cacao and witches' broom disease. Trinidad: Guardian Commercial Printers.

Rigaud, J. Escribano-Bailon, M.T. Prieur, C. Souquet, J.M. and Chaynier, V.

(1993). Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds. *Journal chromatography* A, v. 654, n.2. p. 255-260, 1993.

Roivainen, O. (1969). Effect of vector feeding on virus transmission. *Rep. Cocoa Res. Inst. Ghana.* 1967-68: 40, 1969.

Roivainen, O. (1976). Transmission of cocoa viruses by mealy bugs (Homoptera: Pseudococcidae). *Journal of the Scientific Agricultural Society of Finland* 48, 203-304.

Sahelian Ray, M.D. (2005). Polyphenols, www. aysahelian.com/polyphenols. Polyphenol Research Update: polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr.* 2005 Jan; 81(1):317S-25S.

Serkedjieva, J. and Ivancheva, S. (1999). Antiherpes virus activity of extracts from the medicinal plant *Geranium sanguineum* L. *J. Ethnopharmacology*, 64, 1:59-68.

Sharma, P.N. and Chowfla, S.C. (1991). Some metabolic changes in *Amaranthus caudatus* L. infected with Amaranthus Mosaic Virus. *Plant Dis. Res.*, 6, (1):61-62.

Smith, K. (1937). *A. textbook of plant virus diseases*. London: Churchill Ltd.

Strickland, A.H. (1950). The dispersal of pseudococcidae (Hemiptera-Homoptera) by air currents in the Gold Coast. *Proceedings of the Royal Entomological society London, 25* 1-9.

Suresh, G.L. Govinda-Rao, N. and Gopal-Singh, B. (1991). Physiological changes in French bean infected by Bean Common Mosaic Virus. *J. Res. APAU*, 19, 2:79-81.

Thresh, J.M. (1958). Virus research. In: *Annual Report of the West African*

Cocoa Research Institute. 1956/1957. Page 71.

Thresh, J.M. (1988). Eradication as a virus disease control measure. In B.C. Clifford and A. Lester (eds): *Control of plant diseases: Costs and benefits*, pp 155-194. Blackwell Scientific Publications, Oxford.

Thresh, J.M. (1991). The ecology of tropical plant viruses. *Plant Pathol.* 40:324-339.

Thresh, J.M. and Tinsley, T.W. (1959). The viruses of cocoa. *West African Cocoa Research Institute, Technical Bulletin* no. 7, Crown Agents Overseas Govt. Admin. 32pp.

Thresh, J.M. Owusu, G.K. Boamah, A. and Lockwood, G. (1988). Ghanaian cocoa varieties and Swollen Shoot Virus disease. *Crop Protection* 7: 219:231.

Tinsley, T.W. (1955). The host range of Cacao Viruses: Virus infection in *Adansonia digitata* L. *Rep. West Africa Cocoa Res. Inst.* 1954-55: 33

Tinsley, T.W. and Wharton, A.L. (1958). Studies on the host range of viruses from *Theobroma cacao* L. *Annals of Applied Biology*. 46:1-6.

Titani, K. Koide, A. Hermann, J. Ericsson, L.H. Kumar, S. Wade, R.D. Walsh, K.A. Neurath, H. and Fischer, E.H. (1977). Complete amino acid sequence of rabbit muscle glycogen phosphorylase. *Proc. Natl. Acad. Sci. USA*. 74:4762- 4766.

Toxopeus, H. (1969). Cocoa breeding: the 1965 trials. In: *Annual Report of the Cocoa Research Institute of Nigeria* 1968/1969. Pages 90-92

Usher, (1974). Dictionary of plants used by man. Constable, London.

Vingerhoets Jan, (1997). The World Cocoa Economy: Perspectives on global

Ivvel. *Memorias del Primer Congreso Venezolano del Cacao y su Industria* ISBN 980-620-56-1, *Depósito Legal:* s.f. 11320003386X

Warner, R.C. (1954). "Egg Proteins," in: *The Proteins*, vol.11a, p435 (Neurath, H. ed Bailey, K. eds) Academic Press, New York.

Weber, K. and Osborne, M. (1969). The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, *J. Biol. Chem.* 244, 4406-4412.

Wharton, A.L. (1959). Black pod disease. Resistance and tolerance. *Ann. Rep. West African Cocoa Res. Inst.* 1957/58, 25-30.

Wharton, A.L. and Adams, S.N. (1955) Virus Research: Effect of host plant nutrition. *Report of the West African Cocoa Research Institute for* 1954-55, pp. 36-38.

Winks, M. and Schimmer, O. (1999). Modes of action of defensive secondary metabolites. Function of plant SMs and their exploitation in biotechnology. *Annual Plant Reviews*. Pp. 17-133. Academic Press, Sheffield.

Wood, G.A. R. (1979). Manufacturers needs with respect to quality. *Cocoa Growers Bulletin*, 28:4-6.

World Investment News Multimedia Information Company, (2002). Electronic Edition of the *Special report on Ghana published in Forbes Global Magazine*. Feb 14, 2002 issue. Far Eastern Economic Review. Developed by Agencia.tv, El Poder De La Comunicación.

Wu, Y.Y. and Scheraga, H.A. (1962). Studies of Soybean Trypsin Inhibitor. 1. Physiochemical Properties. *Bio-chemistry*, pubs acs.org. 1.698.

APPENDIX 1

Analysis of variance table for number of CSSV symptoms

Sources	d.f	Sum of squares	Mean square	F value	Prob
Variety (A)	9	2307.006	256.334	3549.2393	0.0000
Days (B)	1	74298.050	74298.050	1028742.2308	0.0000
Var/Day (AB)	9	2787.006	309.667	4287.7009	0.0000
Error	40	2.889	0.072		
Strain (C)	2	25966.633	12983.317	133542.6857	0.0000
Var/Strain (AC)	18	5936.478	329.804	3392.2730	0.0000
Day/strain (BC	2	15056.033	7528.017	77431.0286	0.0000
Var/Day/Str(ABC)	18	5239.078	291.060	2993.7587	0.0000
Error	80	7.778	0.097		
Total	179	131600.950			

Coefficient of Variation: 0.94%

APPENDIX 2

Analysis of variance table for total phenolics content of infected cocoa beans

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Variety (A)	9	3837.114	426.346	33497915.6107	0.0000
Strain (B)	2	4526.260	2263.130	177813617.4321	0.0000
AB	18	7535.880	518.660	32894024.7633	0.0000
Error	60	0.001	0.000		
DAYS (C)	1	3907.926	3907.926	307044916.4555	0.0000
AC	9	1673.744	185.972	14611741.5216	0.0000
BC	2	7055.479	3527.740	277173725.5134	0.0000
ABC	18	2591.204	143.956	11310572.9483	0.0000
Error	60	0.001	0.000		
Total	179	31127.609			

Coefficient of Variation: 0.01%

APPENDIX 3

Analysis of variance table for nitrogen content of cocoa leaves.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.000	0.000	2.0544	0.1374
Variety	9	3.550	0.394	5652.7753	0.0000
Strain	2	18.888	9.444	135360.1673	0.0000
AB	19	4.653	0.258	3705.00073	0.0000
Error	58	0.004	0.000		
Total	89	27.095			

Coefficient of Variation: 0.45%

APPENDIX 4

Interaction between cocoa types and methods of inoculation in severity of Cocoa Swollen Shoot Virus disease

Cocoa types	1st FL	2nd FL	3rd FL	1st FL	2nd FL	3rd FL
	Mealy bug inoculation			Patch graft inoculation		
T85/799 X T65/238	14	33	57	222	241	257
T85/799 X T65/326	52	63	87	117	141	154
T85/799 X PA7	14	36	36	105	179	179
T63/967 X IMC60	24	31	39	77	187	189
T63/967 X T17/524	30	30	49	6	234	250
T63/967 X T65/326	22	21	31	45	213	216
PA150 X NA33	20	27	47	51	165	171
T85/799 X AMEL	12	22	61	79	118	232
AMEL X AMEL	30	42	40	56	222	234
T85/799 X T79/501	22	32	66	60	207	229

APPENDIX 5

Incidence of Cocoa Swollen Shoot Virus disease in mealy bug inoculated seedlings and patch grafted Seedlings.

Cocoa types	1st FL	2nd FL	3rd FL	1st FL	2nd FL	3rd FL
	Mealy bug inoculation			Patch graft inoculation		
T85/799 X T65/238	6.7	20	30	65	65	77
T85/799 X T65/326	38.3	40	53.3	36	60	72
T85/799 X PA7	13.3	23.3	28.3	56	80	82
T63/967 X IMC60	13.3	23.3	23.3	5.6	55.8	60
T63/967 X T17/524	11.7	20	26.7	10	85.7	89
T63/967 X T65/326	11.7	18.3	21.7	5.6	35.2	40
PA150 X NA33	25	40	41.6	30	94	94
T85/799 X AMEL	18.3	35	43.3	5	50	55
AMEL X AMEL	21.7	28.3	40	10	72	75
T85/799 X T79/501	15	26	38.3	1.7	18.5	30

APPENDIX 6

Page gel protocol

12% 1.5 M Tris, pH 8.8 resolving gel protocols for single gel

Substance	Amount
Distilled H_2O	3.35 ml
0.5 M Tris-HCL, pH 8.8	2.5 ml
10% (w/v)SDS 0.1 ml	
Acrylamide/Bisacrylamide (30%/0.8w/v)	4.5 ml
10 %(w/v) ammonium persulfate	0.05 ml
TEMED	0.005 ml
Total	10.005 ml

4.0% 0.5 M Tris, pH 6.8 stacking gel protocols for single gel

Substance	Amount
Distilled H_2O	6.1ml
0.5 M Tris-HCL, pH 6.8	2.5 ml
10% (w/v) SDS	0.1 ml
Acrylamide/Bisacrylamide (30%/0.8w/v)	1.3 ml
10 %(w/v) ammonium persulfate	0.05 ml
TEMED	0.01 ml
Total	10.06 ml